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Nonreciprocal partial cross-incompatibility in maize

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Nonreciprocal partial cross-incompatibility
in maize

by

Kitisri Sukhapinda

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
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Iowa State University
Ames, Iowa

1981

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I. INTRODUCTION

Incompatibility, a condition in which viable pollen grains fail to function in the fertilization of functional eggs, is a common phenomenon occurring in crop plants. Incompatibility is of major interest to plant breeders because it imposes a limitation on selfing and possible recombination. Most studies have been aimed at breaking down the incompatibility system rather than utilizing the system in a practical breeding program. Nevertheless, several successful schemes (Leffel, 1963; Duvick, 1966) have been proposed in order to abet the use of the incompatibility system as a tool in plant improvement programs.

In maize, a number of cases of cross-incompatibility have been reported. Nelson (1952) found that nonreciprocal cross-incompatibility (sterility) occurred in crosses between certain popcorn varieties and sweet corn or field corn. This incompatibility reaction has been attributed to the action of specific gametophytic factors (Gal^S and gal).

A case of cross-incompatibility in maize was recently observed in 1975 among crosses involving controlling elements. When the al-m(pa-pu) Sh/al sh derived lines were crossed by al et/al et males, ears with reduced seed-set (RSS) were produced. In the reciprocal cross (al et/al et females x al-m(pa-pu) Sh/al sh derived males), ears with normal seed-set were obtained.

The etched (et/et) genotype is a participant in this incompatibility reaction. On the basis of reports by Rhoades (1961) and Cox (1966), the et allele is identified with zygotic lethality. This zygotic lethality results in the aberrant transmission of the et allele, but the involvement of the et allele in cross-incompatibility has not been reported.

The objectives of this study include two features: the investigation of the characteristics of cross-incompatibility arising in the controlling element lines involving the cross al-m(pa-pu) Sh/al sh derived lines x al et/al et, and the examination of possible genetic factor(s) controlling the incompatibility reaction in an attempt to establish the inheritance pattern of this character.

II. LITERATURE REVIEW

A. General Review of Self- and Cross-incompatibility in Plants

In plants, incompatibility is usually referred to as a condition in which functional pollen grains fail to participate in the fertilization of functional eggs. There are two types of incompatibility. One type, self-incompatibility, is found more frequently than the second type, cross-incompatibility. Numerous authors have ascribed the role of self-incompatibility as that of preventing self-fertilization which can rapidly lead to inbreeding depression (Allard, 1960).

In general, the genetic control of incompatibility usually includes a series of multiple alleles of the S locus (Arasu, 1968; Frey, 1972). Several authors (Lewis and Crowe, 1958; Wright, 1960; Mayo, 1966) attribute the presence of large numbers of S alleles to mutation.

There are two systems of self-incompatibility: the gametophytic system and the sporophytic system.

1. Gametophytic system

The gametophytic system of incompatibility was studied in Nicotiana by East and Mangelsdorf (1925). They proposed the S-allele hypothesis which held that a pollen grain that contains S alleles identical to one of those in the styles

will not be functional on those particular styles. For example, neither S1 nor S2 pollen functions on S1 S2 styles, whereas S3 pollen is functional. Consequently, the genotypes of the resultant F_1 progenies of the cross S1 S2 by S1 S3 males are S1 S3 and S2 S3. The S1 S1 and S1 S2 genotypes are missing because of the gametophytic incompatibility between the S1 pollen and the S1 S2 styles.

Another type of gametophytic incompatibility was observed in the grass family (Gramineae). To explain the incompatibility occurring in this family, Lundqvist (1954, 1965) proposed a 2-loci system, S and Z. For incompatibility to occur, the alleles at each locus must match in both pollen and style. The two loci act independently but cooperatively, resulting in a unique specificity for each pair of alleles. For example, S1 Z2 pollen is functional in S1 S2 Z1 Z3 style because only the S1 allele of the pollen matches the S1 allele of the style. The Z2 allele of the pollen does not match the Z1 or Z3 alleles of the style. The same S1 Z2 pollen is not functional in S1 S2 Z1 Z2 style because both S1 and Z2 alleles of the pollen match the S1 and Z2 alleles of the style.

Lundqvist (1965) found no evidence for epistasis or dominance interaction between alleles of the same locus in polyploidy. He concluded that the Z locus originated from the S locus by duplication and the complementary reaction evolved.

The 2-loci gametophytic system found in Physalis ixocarpa (Pandey, 1957) is similar to the 2-loci system of Gramineae except that the epistatic reaction between the 2-loci could be observed and incompatibility occurred with matching alleles of either one or both loci. The 2-loci gametophytic system of incompatibility has been reported in other species such as Phalaris coerulescens, Festuca pratense and Beta vulgaris (Frey, 1972).

The 2-loci system demands that both pollen alleles must match those in the styles for incompatibility to occur. As a consequence, a high frequency of homozygosity at 1 locus is generally evident. According to Lundqvist (1958), no deleterious effect was associated with allelic homozygosity in the species having the 2-loci system; whereas, in the species having 1-locus system, homozygosity usually led to reduced plant vigor. Lundqvist (1954) hypothesized that the 2-loci system would give more compatible breeding combinations than the 1-locus system and the restriction on random mating would be less.

In the gametophytic incompatibility system of the Trifolium family, there exists a unique Sf allele. The Sf allele reacts differently from other alleles, Sx (x = 1,2,3...) in the multiple allelic series. The Sf pollen grains can function in any style regardless of the genotype of the style. This was confirmed by Atwood (1945), who demonstrated that the Sf allele has a dominance effect in

the styles, and the Sx pollen grains are functional in the Sf Sx styles. Crowe (1955) found that the Sf allele effect could also be influenced by cytoplasmic factor.

2. Sporophytic system

The sporophytic system of incompatibility was discussed by Gerstel (1950) for guayule (Parthenium argentatum). The essence of this sporophytic system is that the incompatibility reaction depends on the genotype of the pollen parent and not the genotype of the pollen grain itself, and all the pollen grains from one plant act similarly.

Gerstel (1950) postulated that a series of multiple alleles, namely, R1, R2, and R3, does exist in guayule. These R alleles are different from the S alleles of the gametophytic system. Since the incompatibility reaction of the pollen is under the control of the diploid pollen parent, it is apparent that dominance must play a role. Gerstel (1950) also found reciprocal differences in compatibility among certain crosses. He suggested, therefore, that dominance was expressed only in the pollen and not in the style as shown in Figure 1.

The dominance in the pollen, but not in the style, of R2 over the other alleles and of R1 and R3 over R4 would explain the reciprocal differences in certain crosses. For example, the cross R1 R4 female x R1 R2 male (Figure 1) is compatible because all the pollen grains from R1 R2 plant

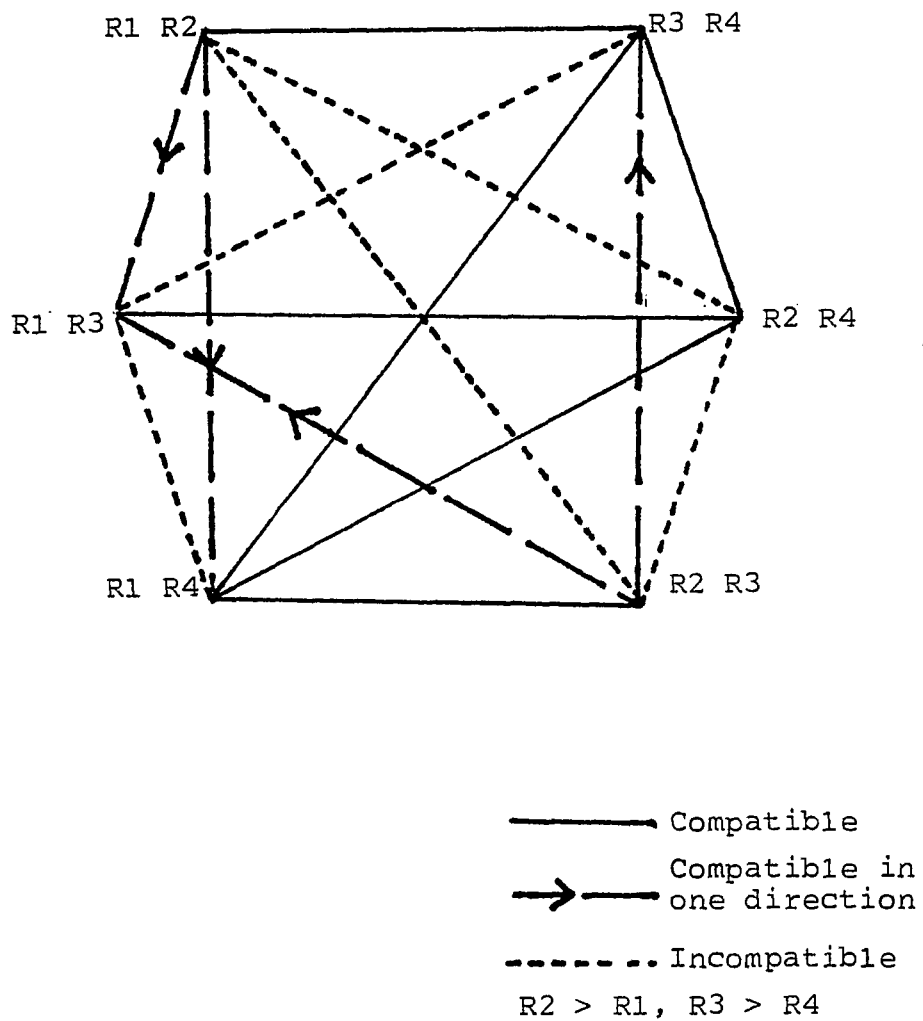


Figure 1. Sporophytic system of cross-compatibility and incompatibility in guayule (Parthenum argentatum) (developed from Gerstel, 1950)

act like R2 pollen, since R2 is dominant to R1 in the pollen parent. However, in the reciprocal cross where pollen grains from R1 R4 plant are crossed on to the R1 R2 styles (Figure 1), all pollen grains from R1 R4 plant act like R1 pollen since R1 is dominant to R4. The R1 allele in the pollen matches the R1 allele in the style; therefore, the cross is incompatible.

Brewbaker (1955) described the sporophytic system of incompatibility in other species including those of the Compositae and Cruciferae families such as Cosmos bipinnatus and Iberis amara.

3. Gene models

The structure and nature of the S gene of the gametophytic system has been the subject of studies by several researchers. Lewis (1965) and Ascher (1966) proposed gene models to explain general gametophytic incompatibility. Lewis (1965) hypothesized that identical polypeptide molecules are produced in the pollen and style by the S gene complex. Each S allele has its own unique molecules. The two molecules or monomers polymerize into dimers in both pollen and style. Applying this model to an incompatible cross, it would be expected that a tetramer is formed by allosteric polymerization of the identical dimers from pollen and style. According to Lewis (1965), the tetramer acts as a genetic regulator for the induction of auxin-inhibitor

synthesis.

Ascher's model (Ascher, 1966) is very similar to Lewis's model. He modified Lewis's model by considering active components from pollen and style to be monomers, which, following an incompatible mating, become dimers through polymerization.

4. Unilateral incompatibility

Unilateral incompatibility has been reviewed by Townsend (1973). The term "unilateral incompatibility" is considered the same as the term "hybridization incompatibility" used to describe the condition in which a species hybrid can be made in only one direction. For example, the pollen of a self-incompatibility (SI) species can function in the style of a self-compatibility (SC) species, but, in the reciprocal cross, pollen of the SC species cannot function in the style of the SI species.

According to Lewis and Crowe (1958), several species of the families Crucifereae, Solanaceae and Onagraceae exhibit unilateral incompatibility (UI). These scientists suggested that the pollen tube growth was restricted in the SI x SC crosses but not in the SC x SI crosses. They observed that some SC species behaved like some SI species; when these SC species were used in the SC x SC crosses, these crosses were incompatible. Consequently, Lewis and Crowe assumed that all SC species originally evolved from SI ancestors through 2

intermediate steps as follows: $SI \rightarrow Sc \rightarrow Sc' \rightarrow SC$. The SC species that behave like SI in UI crosses are considered to be in the Sc stage where Sc pollen is not inhibited by Sc styles or styles with other alleles. The Sc styles reject SC pollen but accept SI pollen. The Sc' style does not inhibit Sc pollen and Sc' pollen is not inhibited by SI style. However, a representative of the Sc' stage has not yet been found.

Pandey (1968) reported that the site of the inhibition of pollen tube growth for the UI matings in interspecific crosses of Nicotiana spp. was in the stigma because the pollen tube growth ceased at the stigma. From this finding, Pandey concluded that the UI phenomenon was under the control of an S gene complex. This S gene complex represents a cluster of genetic elements controlling the physiology of pollen germination and pollen tube growth. According to Pandey, the incompatibility reaction of the S gene complex could be under the influence of polygenes in such a way that the S alleles showed little effect and other nonallelic modifier genes then expressed themselves.

After an extensive investigation on the SI and UI systems in Lycopersicon spp., Martin (1967, 1968) concluded that UI and SI are controlled by the same genetic system; the compatibility reaction of the cross SC x SI in the UI matings is under the control of 2 or more major genes from the SI parent, and the strength of the incompatibility

reaction is influenced by polygenes.

5. Pseudo-self-compatibility

Pseudo-self-compatibility (PSC) is an infrequent phenomenon that occurs in a number of species including Trifolium hybridum (Williams, 1951) and Secale cereale (Lundqvist, 1958). PSC allows self-seed-set by normally self-incompatible plant, resulting in a few homozygous S_x S_x or S_y S_y (x = 1, 2, 3, ..., y = 1, 2, 3, ...) genotypes. The intensity of PSC measured in the form of percent of seed set is under the influence of many factors including variability among S alleles in the ability to allow the pollen tubes to grow in the incompatible styles, genetic modifiers, polyploidy, and the disturbance of the polygenic basis of the incompatibility (Leffel, 1973; Townsend, 1973).

Atwood (1942) suggested that PSC was quantitatively inherited, but Williams (1951) believed that PSC was under the control of the S (self-incompatibility) alleles since a relationship exists between cross-incompatibility and PSC in rye, and the PSC in white clover is a predictable trait.

PSC can also be influenced by an environmental factor such as temperature. Leffel (1963) showed that by increasing the temperature regime, the percentages of self-seed-set for 4 red clover clones increased.

6. Temperature effect

For many years, temperature has been considered to be an important factor affecting compatibility and incompatibility in plants. Generally, relatively high temperatures change incompatibility to compatibility. The site of change is usually in the style and not in the pollen (Townsend, 1973).

The optimum temperature for pollen tube growth of compatible and incompatible crosses varies with species. The temperature at 40°C reduces incompatibility in the style of Trifolium pratense (Kendal, 1968). In Oenothera organensis, the incompatible pollen tube growth is best at 15°C, but is retarded at 20°C. The compatible pollen tube growth is best at 25-30°C (Lewis, 1942).

Dane and Melton (1973) found that in alfalfa (Medicago sativa) pollen germination and pollen tube growth in incompatible matings depended on the temperature at which the plants were grown. At 32°C, the pollen germination decreased when it is compared to 21 and 27°C.

Studies on alsike clover (Trifolium hybridum) by Benner and Townsend (1973) show that temperature treatments have variable effects on different S alleles. Some S alleles are very sensitive to relatively high temperature. These sensitive alleles may lose their function at 32°C and the incom-

patibility reaction will change to the compatibility reaction.

B. Gametophyte Factors in Maize

Gametophyte factors in maize were first observed by Jones (1924). He reported that the F_2 s from the cross between sweet corn and certain popcorn varieties produced less than the expected 25% of sugary kernels. Jones postulated that the scarcity of sugary kernels was due to the competitive effect of a gametophyte factor, Gal vs gal, which is linked to the sugary gene (su) on chromosome 4.

Mangelsdorf and Jones (1926) found that the competitive effect between the dominant (Gal) and the recessive gal pollen is operative only on Gal Gal or Gal gal silks, but not on gal gal silks. In a competitive condition (Gal Gal or Gal gal silks), Emerson (1934) found that only a small percentage (0-4%) of gal pollen effect fertilization.

In addition to the competitive alleles, Gal and gal, a third allele, Gal^S (super gametophyte factor) was described by Schwartz (1950). Schwartz found that Gal^S pollen was the most competitive among the three genotypes in effecting fertilization in the female carrying either Gal^S or Gal. In the gal gal silks, neither Gal^S nor Gal exhibits a competitive advantage over gal pollen.

In order to explain the behavior of differential fertilization of Gal vs gal male gametes in a particular female, Mangelsdorf and Jones (1926) proposed that Gal pollen tubes

grow faster than gal pollen tubes. This faster pollen tube growth rate results in an advantage of Gal gametes in fertilization. This idea was tested by using a linked marker gene for defective seed character, del. If Gal pollen tubes compete favorably in the self-pollination of the genotype del Gal/De1 gal, the linked del character should appear in a higher proportion than the De1 normal character. The resultant ears did show an excess of defective seeds and a higher percentage of defective seeds developed in the lower halves (base) than the upper halves (apex) of the ears. Since the silks at the lower halves of the ears are longer than the silks at the upper halves of the ears, pollen tubes have to grow longer to reach the ovaries in the lower halves of the ears. Thus, Gal del pollen tubes not only grow faster but also longer than gal De1 pollen tubes.

Nelson (1952) considered several possibilities to explain the mechanism of cross-sterility (i.e., gal pollen on Gal^S Gal^S silks). One possibility is that the gal pollen tubes grow rapidly until the pollen reserves are exhausted. At that time, the growth of pollen tube must depend on the silk reserves alone. This subsequent growth of pollen tubes depends upon the interaction between the genotypes of the silk and the pollen. Nelson suggested that the gal pollen tube growth ceases because it cannot utilize the Gal^S Gal^S silk reserves. Another possibility suggested by Nelson holds that an antibody is formed in response to an antigen produced

by the gal pollen tube. This response leads to an antigen-antibody reaction causing the pollen tube to stop growing in the Gal^S Gal^S silk.

Nelson (1952) found that most field corns and sweet corns carry gal allele whereas most popcorns carry Gal allele or the Gal^S allele.

In addition to the Gal locus, several other gametophyte factors have been reported (Coe and Neuffer, 1977). Brieger (1938) found that the aberrant transmission of brittle-1 on chromosome 5 was due to the linked ga2 allele. Longley (1961) found that a case of differential fertilization which occurred in his material was due to the interaction of two independent factors: a ga factor carried by the pollen and a second factor carried by the female plant. The second factor could prevent all or part of the ga pollen from effecting fertilization of the eggs. The action of the female factor could be modified by one or more genetic modifiers or environmental factors.

Rhoades (1943, 1948) discovered a gametophyte factor ga7 located on chromosome 3. The ga7 locus is 6 recombination units distal to et locus. In a competitive condition, the transmission of ga7 is greatly impaired. Only 2 to 8% of ga7 gametes effect fertilization when equal amounts of Gal7 and ga7 pollen are present. The competition occurs on any female regardless of the genotype of the stylar tissue.

A current listing of gametophyte factors is presented in

Maize Genetics Cooperative Newsletter, 1979. The list includes ga8 on chromosome 9 (Bianchi and Parlavecchio, 1965) and ga10 on chromosome 5 (Gonella and Peterson, 1975). The effects of these factors on the functioning of individual pollen grain can be detected only by the differential transmission of linked markers. The wx locus usually is used for ga8, whereas the a2 locus is used to follow ga10.

C. The Etched (et) Allele and the Irregularity of Its Transmission

The et allele is a recessive allele located on the long arm of chromosome 3, 12 units distal to the a1 locus. It is an x-ray induced mutant discovered by Stadler (1940). The allele has pleiotropic effects. A homozygous et et individual is virescent at the seedling stage and the etched kernels have a scarred and pitted appearance. The pericarp at the pitted area is separated from the aleurone layer leaving an air space. The endosperm cells of the scarred area are completely void of starch grains while the normal cells of the surrounding area are packed with starch (Greenblatt, 1962).

Rhoades (1961) found that the etched genotype was associated with a phenomenon that he referred to as "semi-zygotic lethality". He observed an aberrant ratio of A1 (colored): a1 (colorless) kernels from the cross A1 Et/a1 et females by a1 et/a1 et males. Since the transmission of et pollen was normal and the et megaspores were fully viable, Rhoades

attributed the deficiency of colorless or etched kernels to the early elimination of the developing kernels with the et et genotype. The degree of elimination varied with the genetic background.

Cox (1966) concluded from his study that the elimination of etched individuals was due to a modifier of etched genotype, \underline{M}^{et} . This \underline{M}^{et} is located in a position independent of et. According to Cox, the full-strain plants ($\underline{M}^{et} \underline{M}^{et}$ genotype) when used as female plants in the cross A1 Et/a1 et ($\underline{M}^{et}/\underline{M}^{et}$) x a1 et/a1 et (+/+), yield ears totally devoid of etched kernels. However, the same female plants when crossed by a1 Et/a1 Et (++) yield a normal 1:1 colored: colorless ratio. The reciprocal crosses (when the full-strain plants are used as pollen parent) always produce normal 1:1 ratio. Cox established that the distortion of the 1:1 ratio was caused by early elimination of etched individuals and this "semi-zygotic" elimination process was conditioned by \underline{M}^{et} .

Greenblatt (1962) offered a possible biological explanation for this "semi-zygotic" elimination of the etched genotype. He suggested that during endosperm development, the leucoplasts in each endosperm cell becomes defective or lost. The absence of functional leucoplasts leads to an inadequate supply of starch which is essential for the development of the zygote or the embryo.

D. Pollen Germination and Pollen Tube Growth

Pollen germination processes begin immediately after the pollen grain contacts the silk hair or the silk body. Silk exudate is absorbed by the pollen (Pfahler, 1978). The pollen tube emerges from the pore and penetrates the silk hair or the silk body. The tube then reaches the transmitting tissue (Rosen, 1973) surrounding the vascular bundles and grows toward the ovary located at the basal end of the silk. The tube enters the ovary and reaches the embryo sac through the micropyle. Upon entering the embryo sac, the end of the tube ruptures and two sperm nuclei are released. One sperm nucleus fertilizes the egg to form a 2n zygote and the other sperm nucleus fertilizes the two polar nuclei to form 3n endosperm.

The completion of the sequence of pollen germination, pollen tube growth and fertilization requires approximately 25 hours (Pfahler, 1978). Five to 10 hours are required for the 3-cm of tube growth in the distal end of the silk (Walden, 1967). Several factors such as the genotypes of the pollen and the stylar tissue, the length of the silks or the time of pollination may influence the rates of these processes.

Sprague (1933) found that wx pollen grains are slower to germinate and establish themselves on the silks than Wx pollen grains, but the pollen tube growth rates are the same

for the two genotypes. The differences are more pronounced for the grains carrying su wx vs Su Wx. In in vitro studies, Gorla and his colleagues (Gorla et al., 1975) observed great variability in pollen tube growth rates of 20 inbred lines. In analyzing the frequency distribution of pollen tube length for pairs of inbred lines and their F_1 s, they found that the variance of the length of F_1 pollen tubes was greater than the variance of the two inbred parents. They suggested that the segregation of genetic factors influencing the pollen tube growth occurred in the F_1 plants.

The difference between in vitro and in vivo pollen tube growth is evident, presumably because pollen-style interactions are absent in in vitro. The interaction of the pollen-grains and the silk during germination and silk penetration is not well understood. Various chemical substances including enzymes capable of degrading starch to glucose and maltose have been extracted from silk exudate (Martin and Brewbaker, 1973). These chemical substances may interact with the chemical substances that diffuse from the pollen (Stanley, 1973) and give rise to incompatibility interactions.

With compatible pollinations, the pollen metabolizes available substrates for the growth of pollen tube. Large amounts of nutrients and energy are required for the growth of the pollen tube inside the silk. Since the pollen grain itself contains a limited supply of nutrients, pollen tube growth must depend on the silk tissue to provide substrates

for energy metabolism as well as specific structural components and other nutrients which are essential for the development of the pollen tube (Pfahler, 1978).

The interaction between genotypes of pollen and silk tissues which results in differential fertilization ability among different pollen types (Gorla et al., 1976) may, in turn, be due to the differential ability of pollen to utilize the products released by the style.

The pollen tube shows a positive directional growth response to the stylar tissue. Possibly, the transmitting tissue physically guides and directs the pollen tube toward the embryo sac (Rosen, 1964) or chemotropism may play a role in guiding the growing tube (Miki-Hirosige, 1964).

E. Hybrid Dysgenesis in Drosophila melanogaster

The incompatibility reaction found in plant species generally is under the control of simple or complex chromosomal factor(s). The inheritance of these factors such as S gene in Trifolium, Ga gene in maize is well understood.

Incompatibility may also result from more complex factors involving cytoplasmic-chromosomal interaction. Such a phenomenon has been reported in Drosophila melanogaster. An incompatible cross may produce defective F_1 hybrid condition generally referred to as hybrid dysgenesis (Kidwell and Kidwell, 1975; Picard, 1975; Bregliano et al., 1980; Engels and Preston, 1980).

Hybrid dysgenesis is found in certain interstrain crosses in D. melanogaster, especially in crosses between the wild strains and the long-established laboratory strains. Several kinds of abnormalities such as high mutation rate, chromosomal aberration, male recombination, distorted segregation, and sterility may appear in affected individuals (Kidwell et al., 1977; Bregliano et al., 1980).

Two systems of hybrid dysgenesis have been reported: P-M system (Kidwell et al., 1977) and I-R system (Picard, 1975).

1. P-M (Paternal-Maternal) system

A specific dysgenic trait associated with the P-M system is gonadal dysgenesis (GD) which describes the F_1 female sterility resulting from a complete absence of egg-laying (Kidwell and Novy, 1979). GD is caused by the interaction between a chromosomal factor in P strains and a cytoplasmic factor in M strains (Kidwell et al., 1977). GD is found only in the hybrids derived from the cross M female x P male. The hybrids from the reciprocal cross show reduced or no sterility. The M x M and P x P crosses yield normal individuals (Kidwell and Kidwell, 1975; Kidwell et al., 1977).

The studies conducted by Engels (1979) indicate that the chromosomal factors in P strains are polygenic. These factors may be found on all major chromosomes and act independently of each other. Kidwell and Novy (1979) found differences in sterility

in male and female dysgenic hybrids. He proposed that the sex chromosomes, x and y, do not carry the same P factors.

Engels and Preston (1980) investigated the presence of the P factor and M cytotype in strains found in the wild. They reported that the P factor is commonly found in the wild, but the M cytotype is rare. This finding suggests that hybrid dysgenesis occurs infrequently in the natural population. The fact that M cytotype is common only in the long-established laboratory strains leads to two hypotheses concerning the change in frequencies of the M cytotype found in laboratory strains. The first hypothesis which was suggested by Kidwell (1979) held that the marked difference between wild population and laboratory strains was due to the change occurring in wild population. This change probably was caused by the rapid spread of P factors through chromosomal contamination (Picard, 1979). The observed low frequencies of M cytotype probably is a remnant of primordial M population.

The second hypothesis was offered by Engels and Preston (1980) that the cytotype of some laboratory strains has changed from P to M and that the low frequencies of M cytotype in the population probably is an "isolated pocket of M populations" which arises in nature. Engels and Preston also suggested that strong selection favoring reproductive isolation could eventually lead to speciation.

2. I-R (Inducer-Reactive) system

The I-R system of hybrid dysgenesis in D. melanogaster is quite similar to the P-M system. Dysgenic traits in this system are caused by the interaction between the I and R factors. Similar to the P factor in the P-M system, the I factor is quite common in natural population, while the R factor exists only in laboratory strains (Bregliano et al., 1980). The I factor is a chromosomal element controlling inducer condition of male parent, and the R factor is a chromosomal polygenic system controlling reactive conditions of cytoplasm in the oocytes of female parents (Picard, 1975; Bregliano et al., 1980).

The transmission of the I factor is complex according to Picard (1975). He showed that the I factor may be linked to any one of the three major chromosomes and follows strict Mendelian segregation if it is transmitted through heterozygous males. However, if it is transmitted through heterozygous females, the segregation of the I factor deviates from Mendelian ratios. Picard (1979) explained that, in the heterozygous females, a phenomenon called chromosomal contamination occurs and the r chromosomes (reactive originating chromosomes) may acquire an I factor independently of genetic recombination. The contaminated reactive chromosomes which have acquired the I factor behave like the inducer originating chromosomes (i⁺). This change of behavior is irreversible.

Two main hypotheses concern the mechanism of this con-

tamination phenomenon (Bregliano et al., 1980). The first hypothesis implies an insertion of genetic elements in the r chromosome causing contamination of the reactive chromosomes. The second hypothesis is concerned with a derepression of genes carried by all chromosomes but active only on inducer chromosomes. One piece of evidence supporting the first hypothesis has been demonstrated by Pelisson (1978). He showed differences in the ability to induce sterility among the contaminated reactive strains of the same origin. These differences were caused by the differential strength of the inducer chromosomes used in the contamination process. Evidence supporting the second hypothesis is still lacking.

In addition to mutation and nondisjunction, SF sterility (Specific Female sterility) is a dysgenic trait caused by the interaction between the I and the R factors (Picard, 1975; Bregliano et al., 1980). The SF sterility results from failure of some eggs to complete embryonic development. The SF females lay normal quantities of eggs but some of these eggs are defective and do not hatch. The unhatched eggs, if fertilized, will initiate mitosis but the development ceases uniformly between the fifth and eighth cleavage (Picard et al., 1977). The percentage of hatching increases as the age of SF females increases. This unique aging effect is used in the identification of SF sterility (Bregliano et al., 1980).

A comparison of the two hybrid dysgenesis systems,

P-M vs I-R, can be described as follows:

1. Dysgenic traits associated with P-M system occur in both sexes, but those associated with I-R system are restricted to only the females.

2. The behavior of genetic determinants in the two systems is similar. Dysgenesis results from interaction between a chromosomal factor and a cytoplasmic factor in both systems. P factor is comparable to I factor, and M factor is comparable to R factor.

3. Both P and I factors are commonly found in natural populations while M and R factors usually are found in laboratory strains.

4. P-M interaction shows positive correlation with mutation instability (Engels, 1979) while I-R interaction does not show any correlation.

III. MATERIALS AND METHODS

A. Designation of Symbols and Terms

<u>a1</u>	A recessive allele of <u>A1</u> , when homozygous, produces colorless aleurone; not responsive to any regulatory element (elements such as <u>En</u> and <u>Ac</u> that trigger receptive elements so that the locus under control becomes functional)
<u>a1-m</u>	An autonomously mutable allele of <u>a1</u> ; shows spots of color (<u>a1</u> → <u>A1</u>) on colorless background (Peterson, 1961).
<u>a1-m(pa-pu)</u>	A derivative autonomously mutable allele of the original <u>a1-m</u> ; gives pale (<u>pa</u>), purple (<u>pu</u>) and colorless sectors (non-mutation) in colorless background (Peterson, 1970).
<u>a1-m(r)</u>	A colorless kernel allele responsive to <u>En</u> (Enhancer; Peterson, 1960); with <u>En</u> gives colored (purple or red) spots on a colorless background (Peterson, 1961).
<u>a1-m-1</u>	A pale-colored kernel allele responsive to <u>En</u> ; with <u>En</u> gives colored spots on a colorless background (Peterson, 1965).
<u>a1-m(nr)</u>	A colorless kernel allele not responsive to <u>En</u> ; originates as a derivative of the <u>a1-m</u>

	autonomous allele (Peterson, 1970).
<u>En</u>	Enhancer, a regulatory element necessary to trigger mutability at receptive loci; appears at the <u>a1</u> locus or at an independent position (Peterson, 1960).
<u>sh2</u> (or <u>sh</u>)	A recessive allele of <u>Sh2</u> when homozygous recessive, produces shrunken kernels.
<u>et</u>	A recessive allele of <u>Et</u> ; when homozygous recessive gives kernels with scarred, pitted appearance; virescent seedling (Neuffer et al., 1968).
Incompatibility	A term applied for a particular cross between two genotypes that yield a high percentage of ears with reduced seed set.
Compatibility	A term applied to the cross that is in contrast to incompatibility; a high percentage of ears have full or normal seed set.
RSS	A term applied to the appearance of reduced seed set; occurs in an incompatibility cross; the majority of ears produce less than 25 kernels per ear.
NSS	A term applied to the condition that is in contrast to RSS; normal or full seed set; occurs in a compatible cross.
sibbed	Crosses made among plants originated from the same parental crosses.

B. Genetic Stocks

The lines carrying the al-m(pa-pu) allele arose from an al-m autonomously mutable line which in turn originally arose from the pale green mutable stock (Peterson, 1960, 1961). The mutability of the al-m(pa-pu) is recognized by pale (pa), purple (pu) and colorless aleurone sectors on the kernels having colorless background.

The cross-incompatibility between al-m(pa-pu) derived lines and al et tester was first observed in 1975 when al-m(pa-pu) derived lines were used as female parents and al et testers were used as pollen parents. All these al-m(pa-pu) derived lines were developed from a common source (1970 1457 series). The pedigree of this source was traced back to the 1952 561 stock (Table 1). These lines were maintained by outcrossing with al sh/al sh male testers; thus they were in the heterozygous condition, al-m(pa-pu) Sh/al sh. Usually, seeds with pale and purple sectors were selected and used in crosses to maintain the al-m(pa-pu) allele. The lines with the pedigree of al-m(pa-pu) were not intercrossed with the al et/al et tester (Table 1).

When the al-m(pa-pu) derived lines were crossed by pollen from the al et/al et tester, they yielded ears with reduced seed-set (RSS). All the al et/al et lines used were derived from the al et/al et stocks maintained by Dr. P. A. Peterson, Department of Agronomy, Iowa State University. All

Table 1. Pedigree of the al-m(pa-pu) lines; the common source of the incompatible female families, 1970 1457, is traced back to 1952 561 al-m stock; the al-m(pa-pu) genotype was maintained by successive crosses with al sh/al sh males as illustrated

Year	Stock number	Genotype of crosses
1970	1457	<u>al-m(pa-pu)</u> <u>Sh/al sh</u> x <u>al sh/al sh</u>
1966	1438	<u>al-m(pa-pu)</u> <u>Sh/al sh</u> x <u>al sh/al sh</u>
1961	1411-2	<u>al-m(pa-pu)</u> <u>Sh/al sh</u> x <u>al sh/al sh</u>
1960	588-7	<u>al-m(pa-pu)</u> <u>Sh/al sh</u> x <u>al sh/al sh</u>
1959	323-1	<u>al-m(pa-pu)</u> <u>Sh/al sh</u> x <u>al sh/al sh</u>
1957	152A-1	<u>al-m</u> <u>Sh/al sh</u> (open pollinated)
1956	139-5	<u>al-m</u> <u>Sh/al sh</u> x <u>al sh/al sh</u>
1955	21-2	<u>al-m</u> <u>Sh/al-m</u> <u>Sh</u> x <u>al sh/al sh</u>
1954	2-45	<u>al-m</u> <u>Sh/al-m</u> <u>Sh</u> selfing
1953	37-1	<u>al-m</u> <u>Sh/al-m</u> <u>Sh</u> selfing
1952	561	<u>al-m</u> <u>Sh/al-m</u> <u>Sh</u> x <u>al-m</u> <u>Sh/al-m</u> <u>Sh</u>

al et/al et lines appear to give the same results regarding the incompatibility with al-m(pa-pu).

The pedigrees of early and medium al et/al et lines are shown in Tables 2A and 2B. Both early and medium lines were derived from a common source, 1965 279. Sibbed and selfed progenies have been used in maintaining the stocks.

Table 2A. Pedigree of the a1 et tester; the 1975 0501-0510 stock is the common seed source for early lines (early dehiscence)

Year	Stock number	Genotype of crosses
1975	0501-0510	<u>a1 et/a1 et</u> sibbed
1974	0501-0520	<u>a1 et/a1 et</u> sibbed
1970	1101-1131	<u>a1 et/a1 et</u> sibbed
1967g	105A & B15	<u>a1 et/a1 et</u> sibbed
1967	0951	<u>a1 et/a1 et</u> sibbed
1966g	22A	<u>A1 et/a1 et</u> sibbed
1966	0432	<u>a1 et/a1 et</u> x <u>A1 et/A1 et</u>
1965g	32A	<u>A1 et/a1 et</u> sibbed
1965	1411 x 279	<u>A1 et/A1 et</u> x line <u>A1/a1 et</u>
1962	792-25	<u>A1 et/A1 et</u> selfed

Table 2B. Pedigree of the a1 et tester; the 1969 0921-0930 stock is the common seed source for medium lines

Year	Stock number	Genotype of crosses
1969	0921-0930	<u>a1 et/a1 et</u> sibbed
1968	9031	<u>a1 et/a1 et</u> sibbed
1967	3907-3917	<u>a1 et/a1 et</u> sibbed
1966	0401-0420	<u>a1 et/a1 et</u> sibbed
1965	279, 289	<u>a1 et/line A1</u> sibbed
1964	1085	<u>a1 et/line A1</u> sibbed

C. Methods

1. Field experiment

The genotypes of maize plants used in these experiments were grown in successive years at the Iowa State University, Agronomy Research Center in Ames. Crosses were made according to the usual corn genetics crossing procedure; bagging tassels the day before use, shaking the pollen bag over the silks that had been cut back the previous day.

2. Determination of classes of ears with differential fertility resulting from incompatible and compatible crosses

After the mature ears were harvested, the numbers of kernels per ear were counted and recorded. These ears were placed into one of the following three classes according to the level of fertility (Figure 2):

L (low)	ears producing less than 25 kernels per ear
I (intermediate)	ears producing 26 to 200 kernels per ear
H (high)	ears producing greater than 200 kernels per ear

3. Statistical analysis

The data (number of set seed per ear) from all tested crosses were classified and recorded into 3 classes, L, I and H. These ear classes and the genotypes of the crosses



Figure 2. Three classes of ears with differential numbers of seed-set: L (low) = < 25 k/ear, I (intermediate) = 26 to 200 k/ear, and H (high) = > 200 k/ear

were used as 2 classifications in the 2-way or contingency tables. The chi-square test (Steel and Torrie, 1980) was used in testing the independence of the ear classes from the genotypes of the crosses.

To simplify the test, the values of the classes, I and H, were combined and the combined value was tested against class L. Since class L represents the resulting ears of incompatible crosses, it is the class of interest for this study. Meanwhile, the type of crosses (compatible vs incompatible) that produced ears of class I could not be identified with satisfactory certainty. The values of this class were included with the values of class H which represented the results of compatible crosses.

For the contingency table, the null hypothesis and the alternative hypothesis are as follows:

$$H_0 = p_{ij} = p_{i.}p_{.j}$$

$$H_1 = p_{ij} \neq p_{i.}p_{.j}$$

where p_{ij} = probability of a randomly selected individual being classified in i , j th cell; $i = 1 \dots r$,
 $j = 1 \dots c$

$p_{i.}, p_{.j}$ = row and column probability, respectively;

$$\sum_i p_{i.} = 1 = \sum_j p_{.j}$$

The test criterion is

$$\chi^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}} \quad (r-1)(c-1)\text{df}$$

The expected values were calculated under the assumption that the null hypothesis was true. The expected values are given by

$$E_{ij} = \hat{p}_{ij} n_{..} = \frac{n_{i.} n_{.j}}{n_{..}}$$

where E_{ij} = expected value of i , j th cell

\hat{p}_{ij} = estimation of $p_{ij} = \hat{p}_{i.} \hat{p}_{.j}$,

$$\hat{p}_{i.} = \frac{n_{i.}}{n_{..}} \quad \text{and} \quad \hat{p}_{.j} = \frac{n_{.j}}{n_{..}}$$

$n_{i.}$ = total value of i th row

$n_{.j}$ = total value of j th column

$n_{..}$ = grand total of all rows and columns.

4. In vitro pollen germination

a. Preparation of medium The liquid medium was made according to the CWRM (Cook and Walden Revised Medium) formula (excluding the agar) suggested by Cheng and Freeling (1976). This formula includes 17% w/v sucrose, 200 mg/l $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 100 mg/l H_3BO_3 . After heating, the pH was adjusted to 6.4. The medium could be stored at 4°C for several days.

b. Pollen collection A section of tassel was collected one day before anthesis. The cut end of the tassel was put in water to keep the tassel fresh and reusable for a few days. Mature anthers were collected the following day when anthesis occurred.

c. Preparation of slides An anther was split open with forceps and the pollen grains were tapped over the liquid

medium on a depression slide. The slide was kept in a closed petri dish during the time course of pollen tube growth. A series of slides were made since the observation must be made under a light microscope, and the heat from the light usually causes the pollen tubes to burst. Each stage of pollen tube growth was captured by refrigerating one slide every 5 minutes. In this way, the process of pollen tube growth could be observed.

5. In vivo pollen germination

a. Sample collection A number of field grown al-m(pa-pu) plants were pollinated by pollen from al et/al et parents (incompatible type) and a few of the al-m(pa-pu) plants were selfed. Approximately 24 hours after pollination, samples of the silks from these ears were collected and stored in the fixing solution (3 parts ethanol + 1 part acetic acid) for at least 1 hour.

b. Staining procedures The silks were prepared according to Dionne and Spicer's (1957) method in the following manner:

1. Hydrolyze the silks in 45% acetic acid at 60°C for 60 minutes.
2. Stain the silks with staining solution (150 mg Safranin O², 20 mg anilin blue, and 25 ml hot 45% acetic acid) for 5-10 minutes, on a slide.

3. Squash the silk tissues under a cover slip to observe pollen tube growth inside the silk tissues.

IV. RESULTS

A. The Occurrence of Ears with Reduced Seed-Set (RSS) in Specific Crosses

Reduced seed-set (RSS) occurs among unidirectional crosses between lines derived from the al-m(pa-pu) (1970 1457) stock (Table 1) and al et/al et pollen testers derived from al et/al et (1969 0921-0930 and 1975 0501-0510) stocks (Tables 2B and 2A). RSS was first observed in the 1975 field nursery (Table 3A, line 1). In that year, nearly one entire range included derivatives of al-m(pa-pu) stock. [The derivatives of al-m(pa-pu) are those mutable genotypes derived from the al-m(pa-pu) mutable allele, Peterson (1960, 1961, 1970).] These al-m(pa-pu) derivatives were scheduled to be crossed by al et/al et pollen parent to detect the presence of En controlling element (Peterson, 1960). In a range of 60 rows, a total of 45 rows with approximately 10 to 12 plants of al-m(pa-pu) derivatives per row were pollinated by pollen from al et/al et testers. Ears with reduced seed-set were observed in all but two of the 45 rows. (The two exceptional rows were not from the same pedigree as the other 43 rows.) The number of seed-set varied from complete barrenness to greater than 200 kernels per ear. Each ear is placed in one of the 10 classes according to the number of kernels on the ear. A total of 347 ears was obtained from the 1975 crosses. The ear classes and the percentage of total for each class

Table 3A. Observed RSS in the cross al-m(pa-pu) Sh/al sh females x al et/al et males in five consecutive years

1 Year tested	2 Number of ears of each class (%)			5 Total
	L=<25 k/ear	I=26-200 k/ear	H=>200 k/ear	
1 1975	308 (89)	35 (10)	4 (1)	347
2 1976	410 (86)	31 (6)	38 (8)	479
3 1977*	73 (77)	22 (23)	0 (0)	95
4 1978	140 (87)	20 (16)	1 (.6)	161
5 1979	218 (88)	23 (9)	6 (3)	247

*Significant at .05 level.

is given as follows:

Class	Description (Number of kernels/ear)	Percent of total
1	0	76
2	1-25	13
3	26-50	4
4	51-75	2
5	76-100	0.02
6	101-125	1
7	126-150	0.05
8	151-175	0.08
9	176-200	0.08
10	>200	1

The distribution of the frequencies of ears showing different numbers of seed-set per ear is shown in Figure 3.

To simplify the procedure of the identification of incompatible crosses, the different classes were reclassified into three groups: low, intermediate, and high. Classes 1 and 2 were combined as class L (low = <25 k/ear), classes 3, 4, 5, 6, 7, 8, and 9 were combined as class I (intermediate = 26 to 200 k/ear; class 10 represents normal or full seed-set and is identified as class H (high = >200 k/ear)).

Based on these three classes (L, I, and H), the results of 1975 crosses between the al-m(pa-pu) females and al et males are shown in Table 3A, line 1. Approximately 89% of the ears were assigned to class L, 10% to class I, and 1% to class H. As is also illustrated in Table 3A, RSS was evident in repeated crosses of al-m(pa-pu) females and al et males in 1976, 86% were of class L, 6% class I, and 8% class H (Table 3A, line 2).

These particular crosses, al-m(pa-pu) females x al et males were reexamined in 1977, 1978, and 1979. The results (Table 3A, line 3, 4, 5) indicate that 77 to 88% of the tested ears are of class L, while the rest of the tested ears are of class I and H.

According to the 5 year-data (Table 3A), it is evident that the proportion of the ears in class L varied only slightly from year to year (Table 3A, column 2), while the

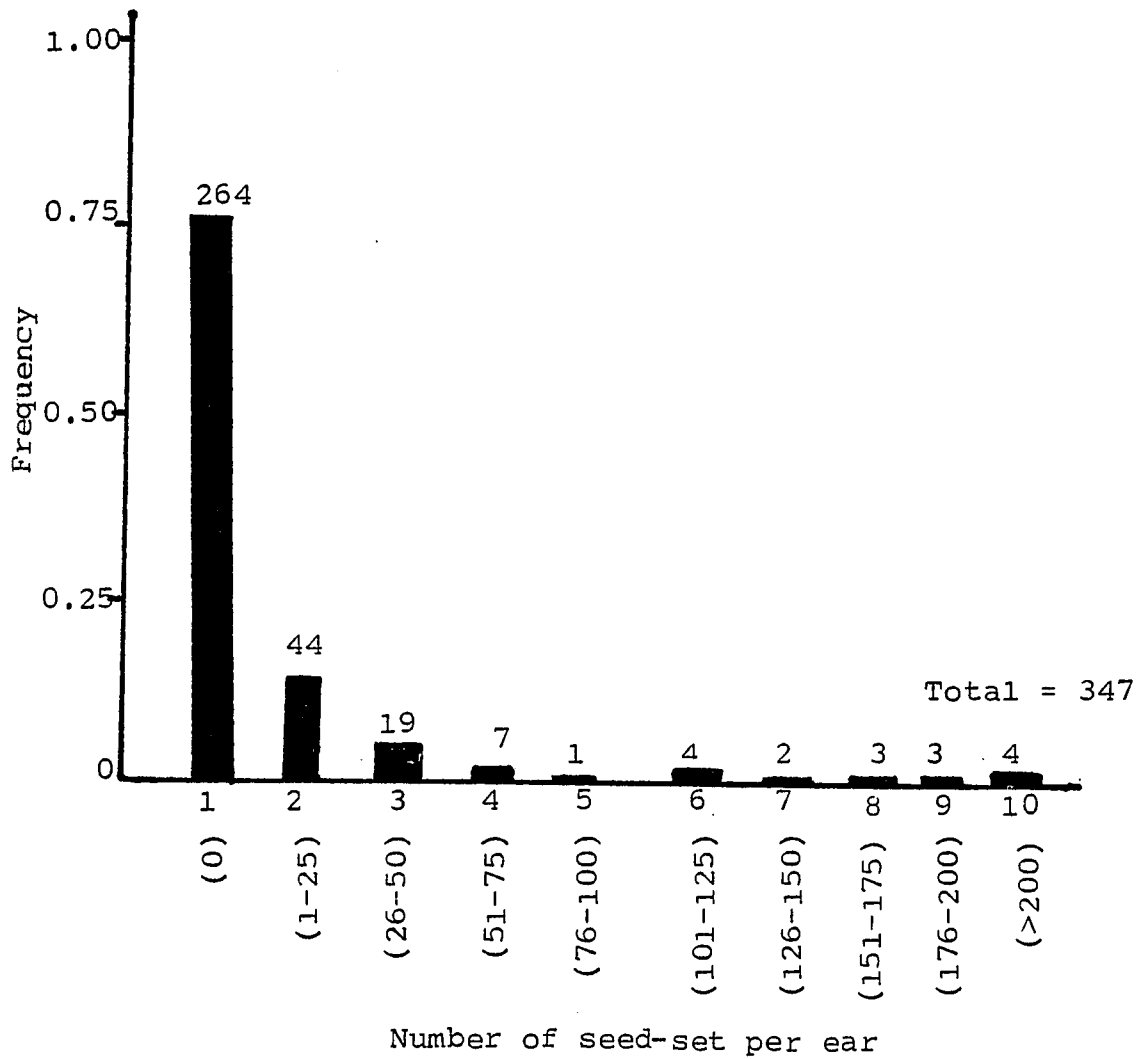


Figure 3. Frequency distribution of 10 classes of ears from 1975 crosses of the derivatives of al-m(pa-pu) Sh/al sh (al-m(nr) Sh/al sh x al et/al et)

proportion of the ears between classes H and I did vary (Table 3A, columns 3, 4).

To simplify the statistical analysis, the values of class I and class H were combined when chi-square testing was conducted (see Materials and Methods).

The 5-year results of the incompatibility tests were compared statistically by chi-square testing (Steel and Torrie, 1980). A general agreement holds that most crosses between al-m(pa-pu) females and al et males express incompatibility interaction by exhibiting RSS effect. It should be pointed out that the RSS effect is determined by the proportion of the ears in class L (<25 k/ear).

Since the incompatible crosses between al-m(pa-pu) females and al et males yield partial seed-set instead of complete sterility, these particular crosses reflect an incomplete incompatibility. The term "incompatibility" hereafter refers to "partial incompatibility".

Though there is a general agreement among the 5-year results in the class distributions (Table 3A), a statistically significant difference at .05 level is detected in the 1977 results. In this year, only 77% of the ears examined were of class L, whereas in the other four years, an average of 87% was observed. This significantly lower-than-average value of class L may have resulted from contamination or mishandling of materials.

Since the al-m(pa-pu) source lines produce ears with RSS

when al et plants are used as pollen parents, the following questions arose: (1) are the al-m(pa-pu) lines particularly prone to produce ears with a low seed-set; (2) is inviable pollen of the al et/al et parent the basis for the RSS effect; (3) is the incompatibility confined to a specific interaction between al-m(pa-pu) and al et; and (4) is the RSS appearance limited to the female al-m(pa-pu) x male al et/al et or does the reciprocal cross also show RSS?

An insight into the first question, that of susceptibility of al-m(pa-pu) for RSS, was gained from the examination of crosses of al-m(pa-pu) females with testers other than al et/al et.

In 1972 and 1974, al-m(r) Et/al-m-1 Et and al sh Et/al sh Et were used as pollen parents with al-m(pa-pu). In 1978 and 1979, crosses were made with an additional source of pollen, Al Sh et/al sh Et (Table 3B). Resulting ears were classified as previously described in the Materials and Methods. In these crosses, the al-m(pa-pu) lines usually produce ears with normal seed-set, indicating that RSS is not caused by a poor seed-setting al-m(pa-pu) line.

According to the chi-square test of the distribution of ear classes using the total values (Table 3B, lines 3, 7, 9), the differences among the three pollen sources in affecting seed-set on al-m(pa-pu) are nonsignificant. The frequency of class L averages 5%. This low frequency of ears with low seed-set is often found in any genetic nursery row and is

Table 3B. Observed seed-set in the cross al-m(pa-pu) Sh/al sh x males other than al et/al et; results are from 4 different years of crosses

1 Genotype of male parent (ns1) ^a	2 Year (ns2) ^a	3 Number of ears of each class (%)			6 Total
		4 L=<25 k/ear	5 I=26-200 k/ear	6 H=>200 k/ear	
<u>al-m(r)/al-m-l</u>	1972	11 (7)	37 (23)	111 (70)	159
	1974	6 (3)	43 (21)	156 (76)	205
	Total ^b	17 (5)	80 (22)	267 (73)	364
<u>al sh/al sh</u>	1972	8 (6)	61 (45)	67 (49)	136
	1974	5 (5)	47 (51)	42 (46)	94
	1978	1 (2)	7 (15)	40 (83)	48
	Total ^b	14 (5)	115 (41)	149 (53)	278
<u>Al Sh et/al sh Et</u>	1979	3 (3)	7 (8)	77 (89)	87
	Total ^b	3 (3)	7 (8)	77 (89)	87

^ans1 = nonsignificant difference among genotypes; ns2 = nonsignificant difference among years.

^bTotal of each genotype.

generally ascribed to imperfect crosses caused by human error or from poor pollen performance such as failure of germination caused by hot weather.

With reference to question 2 on the possible inviability of al et pollen, several lines of evidence invalidate this possibility. When the pollen from al et/al et plants was used on ears of stocks other than al-m(pa-pu), seed-set was generally normal. An example of these particular crosses is shown in Table 3C. The same low frequencies of class L (as compared to those in Table 3B) were observed and these could result from the same factors outlined for the pollen parent tests (Table 3B).

It can be concluded from these two sets of data (Tables 3B and 3C) that al-m(pa-pu) is not a poor seed setter and the al et parent is not a poor pollen producer. Since neither the two parents, al-m(pa-pu) and al et, alone shows RSS effect, the RSS effect must result from an incompatibility reaction between the two genotypes.

Since al-m(pa-pu) was identified as an incompatible female in the cross al-m(pa-pu) x al et/al et, the fourth question arose as to the type of effect (RSS or NSS) that would arise in the reciprocal cross. The test of reciprocity of the incompatibility between al-m(pa-pu) and al et lines was conducted in 1976, 1978, and 1979. The data from these three years were combined since from previous evidence (Tables 3A and 3B) the year effect was not significant. The

Table 3C. Observed seed-set in the cross having female parent (other than al-m(pa-pu) lines) by al et/al et males

	1 Female parent	2 Year tested ^a ns	3 Number of ears of each class (%)			6 Total
			L=<25 k/ear	I=26-200 k/ear	H=>200 k/ear	
1	1970 3835 <u>al-m (dense)</u>	1975	10 (10)	33 (34)	53 (55)	96
2		1978	2 (5)	23 (56)	16 (39)	41
3		Total ^b	12 (9)	56 (40)	69 (50)	137

^ans = nonsignificant difference (between years).

^bTotal of 2 years.

al-m(pa-pu) derivatives from 2 original sources, 1970 1457-2 and 1457-5 (the derivation of these lines will be discussed later on in the Results) were used in the two-way crosses, al-m(pa-pu) Sh/al sh x al et/al et and al et/al et x al-m(pa-pu) Sh/al sh.

The results reported in Table 4 indicate that the incompatibility reaction between al-m(pa-pu) and al et is unidirectional. Incompatibility occurs only when the al-m(pa-pu) lines are used as female parents (cross a) and al et lines are used as pollen parents (Table 4, lines 1 and 3). In the reciprocal cross (cross b), al et/al et females x al-m(pa-pu) Sh/al sh males, the resulting ears show a normal seed set (Table 4, lines 2 and 4). The chi-square tests show two features: the difference between the reciprocal crosses is significant at .01 level, and the difference between the original sources (1970 1457-2 vs 1970 1457-5) of the al-m(pa-pu) lines is nonsignificant.

B. Heritability of the RSS Effect in the al-m(pa-pu) Lines

Based on the evidence shown thus far, it is reasonable to postulate that the al-m(pa-pu) females express a partial incompatible condition when pollinated by a specific source of pollen, namely al et/al et. The incompatibility is incomplete and is now identified as the RSS effect. RSS is a condition where a certain high percentage of the ears has

Table 4. Difference in seed-set between the reciprocal crosses, al-m(pa-pu) Sh/
al sh x al et/al et vs al et/al et x al-m(pa-pu) Sh/al sh, using 2
original al-m(pa-pu) lines

1			3	4	5	6
Original lines		<u>Crosses</u>	<u>Number of ears of each class (%)</u>			
		Female** x male**	L=<25 k/ear	I=26-200 k/ear	H=>200 k/ear	Total
1	0 1457-2	(a) <u>al-m(pa-pu) Sh/al sh</u> x <u>al et/al et</u>	330 (86)	24 (6)	29 (8)	383
2		(b) <u>al et/al et</u> x <u>al-m(pa-pu) Sh/al sh</u>	3 (1)	73 (30)	170 (69)	246
3	0 1457-5	(a) <u>al-m(pa-pu) Sh/al sh</u> x <u>al et/al et</u>	80 (83)	7 (7)	9 (9)	96
4		(b) <u>al et/al et</u> x <u>al-m(pa-pu) Sh/al sh</u>	1 (1)	20 (29)	47 (69)	68

**Significant difference between the reciprocal crosses at .01 level.

less than 25 kernels/ear.

The questions that follow are the questions of genetic control of RSS and its heritability. These questions can be ascertained by following the pedigree of the al-m(pa-pu) lines that show RSS effect. By tracing the numerous crosses of the 1970 1457 source to the ears from which they were derived, the parental contributions could be determined. Seeds from these ears that were progenies of the cross al-m(pa-pu) Sh/al sh x al sh/al sh, 1970 1457-1, 1457-2, 1457-3, and 1457-5, were planted in 1977, 1978, and 1979.

These seeds were subjected to tests with pollen from the al et/al et testers. The resulting ears showed a similar RSS effect (Table 5A, column 4).

In maintaining the al-m(pa-pu) lines, al sh/al sh male parents were used as the recurrent parent. As a consequence of this series of crosses, the al-m(pa-pu) allele was maintained in the heterozygous condition as al-m(pa-pu) Sh/al sh. As evident in the traced pedigree illustrated in Table 5A, al-m(pa-pu) Sh/al sh parent was used as the female parent in all of these backcrosses.

From these successive backcrosses of al-m(pa-pu) Sh/al sh, colorless derivatives (a-m(nr) Sh/al sh; Peterson, 1961) were isolated and used as female parents in crosses with the al-m(r)/al-m-1 male testers in order to test for the presence of En. The derivative progenies of this cross, al-m(nr) Sh/al sh x al-m(r)/al-m-1 with En were then

Table 5A. Origin of derivatives of al-m(pa-pu) lines showing RSS effect when crossed by al et/al et males

1	2	3	4	5
Year of cross	Crosses ^a	Genotype ^b	Result (seed-set)	Year tested
1 1966	<u>al-m(pa-pu) Sh/al sh</u> x <u>al sh/al sh</u>	- ^c	-	-
2 1970	<u>al-m(pa-pu) Sh/al sh</u> x <u>al sh/al sh</u> → <u>al-m(pa-pu)/al sh</u>		RSS	1977, 78, 79
3 (1457-1,2,3)				
4 1971	<u>al-m(pa-pu) Sh/al sh</u> x <u>al sh/al sh</u>	-	-	-
5 1972	<u>al-m(nr) Sh/al sh</u> x <u>al-m(r)/al-m-1</u> → <u>al-m(nr)/al-m(r)</u>		RSS	1974
6		<u>al-m(nr)/al-m-1</u>	RSS	1974
7	<u>al-m(pa-pu) Sh/al sh</u> x <u>al sh/al sh</u>	-	-	-
8 1974	<u>al-m(nr) Sh/al sh</u> x <u>al-m(r)/al-m-1</u> → <u>al-m(nr)/al-m(r)</u>		RSS	1975, 76, 77
9		<u>al-m(nr)/al-m-1</u>	RSS	1975, 76, 77

^a . > = parents to progeny.

^b Confirmed genotypes when tested as female.

^c - = no test.

test-crossed by al et/al et to determine En presence and position (Peterson, 1970). In these crosses with the al et/al et male parents, the resulting ears, again, showed the RSS effect (Table 5A, lines 8 and 9).

From these illustrative crosses (Table 5A), it is evident that the RSS effect in al-m(pa-pu) lines is heritable and, further, it is maintained from generation to generation despite the number of outcrosses with al sh/al sh or al-m(r)/al-m-1 male testers.

It is surprising that the RSS effect seems to be enhanced in the progeny derivatives when this is compared to the original 1970 1457 sources (Table 5B). Two out of three comparisons (Table 5B, lines 1, 2, 3, and 4) show the enhanced RSS effect in the progeny derivatives. Because the RSS effect is enhanced despite several generations of outcrosses by al sh/al sh and al-m(r)/al-m-1 males, it can be concluded that the RSS condition in al-m(pa-pu) lines is not only retained but enhanced by these male parents.

Line 1970 1457-3 represents an exception to the general observation that the RSS effect is enhanced in the progeny derivatives. In this line, no change in the RSS effect occurs (Table 5B, lines 5 and 6). The values of class L persist at 89% for both the originals and the progeny derivatives.

Line 1970 1457-5 could not be used in a strict comparison since the original ear was not available.

Table 5B. Difference between original 1970 sources and their derivatives in the RSS effect within four al-m(pa-pu) lines (test-crossed by al et/al et males)

1		2	3	4	5	6	7
<u>al-m(pa-pu)</u>		Seed source ^a	Table	Number of ears of each class (%)			Total
lines			5A	L=<25	I=26-200	H=>200	
			line	k/ear	k/ear	k/ear	
1	0 1457-1	Originals*	2	3 (17)	10 (56)	5 (28)	18
2		Derivatives*	8,9	49 (43)	56 (49)	10 (9)	115
3	0 1457-2	Originals*	2	13 (68)	1 (5)	5 (26)	19
4		Derivatives*	8,9	330 (86)	24 (6)	29 (8)	383
5	0 1457-3	Originals ^{ns}	2	17 (89)	2 (11)	0 (0)	19
6		Derivatives ^{ns}	8,9	255 (89)	29 (10)	1 (.3)	285
7	0 1457-5	Originals		- -	- -	- -	-
8		Derivatives	8,9	133 (84)	13 (8)	12 (7)	158

^a_{ns} = nonsignificant.

*Significant at .05 level.

That heritable variation in the RSS expression is present in the original population is evident from examining the three original sources, 1970 1457-1, 1457-2, and 1457-3, and the progeny derivatives of four original sources, 1970 1457-1, 1457-2, 1457-3, and 1457-5. In this comparison, the 1970 1457-1 line shows the lowest RSS effect both in the original line and the derivatives (Table 5C). The other three lines, 1970 1457-2, 1457-3, and 1457-5, show a higher frequency of class L (Table 5C). The frequencies of class L of line 1970 1457-1 and its derivatives are significantly lower than those of the same class of the other two original lines (1970 1457-2, 1457-3) and their derivatives (Table 5C, column 3). The values of class L of line 1970 1457-2 and those of line 1970 1457-3 are not statistically different.

It should be noted that the numbers of plants used in the testing of the RSS effect in the original lines were low because of the low percent germination due to the age of the seeds. The few numbers of tested ears might affect the accuracy of the comparative values.

It is also noteworthy that there seems to be an upper limit of the frequency of class L. In a population cross, al-m(pa-pu) Sh/al sh x al et/al et males, class L-type progeny does not exceed 90%. Approximately 80 to 90% of the ears are found to produce less than 25 k/ear (class L).

The 90% level seems to be an upper level where the RSS effect reaches a stable level. This postulation is supported

Table 5C. Difference in RSS effect among al-m(pa-pu) lines; comparison within the original sources (Table 5A, line 2) and the derivatives (Table 5A, lines 8 and 9) when crossed by al et/al et males

1		2	3	4	5	6
Seed source		al-m(pa-pu) lines	Number of ears of each class (%)			Total
			L=<25 k/ear	I=26-200 k/ear	H=>200 k/ear	
1	Original	0 1457-1**	3 (17)	10 (56)	5 (28)	18
2		0 1457-2	13 (68)	1 (5)	5 (26)	19
3		0 1457-3	17 (89)	2 (11)	0 (0)	19
4	Derivative	0 1457-1**	49 (43)	56 (49)	10 (9)	115
5		0 1457-2	330 (86)	24 (6)	29 (8)	383
6		0 1457-3	255 (89)	29 (10)	1 (.3)	285
7		0 1457-5	133 (84)	13 (8)	12 (7)	158

**Significant difference at .01 level.

by the observation that the value of class L of the derivatives of line 1970 1457-3 does not deviate from the value of class L of the original line (89%) (Table 5C). Moreover, a consistency in the frequency of class L of the derivatives line 1970 1457-2, 1457-3, and 1457-5 exists (86-89%) (Table 5C).

C. Genetic Control of the Incompatibility Condition in the al-m(pa-pu) Lines

The incompatibility condition expressed in al-m(pa-pu) lines in the cross al-m(pa-pu) Sh/al sh x al et/al et male is heritable (Tables 5A, 5B, and 5C). It is further evident that this condition is maintained in spite of successive generations of outcrosses by the recurrent male parent, al sh/al sh.

At least four explanations can be considered for the genetic control of this incompatibility condition.

1. The cytoplasm of al-m(pa-pu) may be a factor. In developing al-m(pa-pu) lines, the al-m(pa-pu) Sh/al sh and its derivative genotypes have been used in crosses as the female parent (al-m(pa-pu) Sh/al sh x al sh/al sh or al-m(r)/al-m-1; Table 5A). In such a series of crosses, the incompatibility condition or RSS effect in al-m(pa-pu) lines could be transmitted from generation to generation through the cytoplasm of the al-m(pa-pu) female parents. If the RSS effect is under the control of a cytoplasmic factor, crossing

al-m(pa-pu) lines by al sh/al sh males will not dilute the RSS effect.

2. The second possibility includes the role of the al-m(pa-pu) allele as the determinant role in RSS expression. In each cross [al-m(pa-pu) Sh/al sh x al sh/al sh or al-m(r)/al-m-1; Table 5A], the al-m(pa-pu) allele or derivative allele has been selected and could transmit the incompatibility condition or RSS effect. It could also include a closely linked gene.

3. A simple gametophyte factor such as the ga gene could also control the RSS effect. Specifically, it could only be a Gal^S type allele of the Gal locus on chromosome 5 since only this allele gives a sterility effect (Schwartz, 1950; Nelson, 1952). As an example, in this case, it can be assumed that the RSS effect of the al-m(pa-pu) lines involved the Gal^S allele.

4. The last possibility to explain the RSS effect in al-m(pa-pu) lines is an interaction of a cytoplasmic factor and a chromosomal factor.

1. Test of the role of cytoplasmic factor in the control of the RSS effect

The al-m(pa-pu) lines have been maintained in a heterozygous condition, al-m(pa-pu) Sh/al sh, by backcrossing with al sh/al sh males. These lines have always been used as the female parent. Thus, when the RSS effect is expressed in crosses of al-m(pa-pu) lines by al et/al et males, the

original al-m(pa-pu) cytoplasm is always included (Table 5A).

If the original (1970 1457 series) al-m(pa-pu) cytoplasm contains the proposed RSS controlling factor, and if the RSS factor is specific for the al-m(pa-pu) cytoplasm, then it would be expected that a different cytoplasm but with the same genotype would not express the RSS effect.

To test such possibility of whether the RSS control includes a cytoplasmic component, 3 different cytoplasmic sources were examined. The test was conducted as follows. Ten pairs of reciprocal crosses [al-m(pa-pu) Sh/al sh x al sh/al sh males and al sh/al sh x al-m(pa-pu) Sh/al sh males] were made (Figure 4A, columns 1 and 2). The F_1 s from these reciprocal crosses possess the same genotype [al-m(pa-pu) Sh/al sh], but differ in their cytoplasm [al-m(pa-pu) vs al sh sources]. The F_1 plants were assayed for the RSS effect by testcrossing with al et/al et males. The F_1 s from both crosses (Figure 4A, lines 4 and 5) show RSS effect. From 49 ears with al-m(pa-pu) cytoplasm, 42 ears (86%) fall in class L (Figure 4A). This proportion of class L agrees with that found previously for al-m(pa-pu) derivatives (Table 3A and Table 5B), and served as control. From 26 ears having al sh cytoplasm, only 17 ears (65%) fall in class L. This 65% is somewhat lower than the control. However, when the phenotype of the seeds on these ears was examined, shrunken seeds were segregating on a few ears. These shrunken seeds must have been derived from self-

1	<u>al-m(pa-pu)</u> ¹ cytoplasm	<u>al sh</u> ² cytoplasm
2	<u>al-m(pa-pu)</u> <u>Sh/al sh</u> x <u>al sh/al sh</u>	<u>al sh/al sh</u> x <u>al-m(pa-pu)</u> <u>Sh/al sh</u> (10 crosses)
3	<u>al-m(pa-pu)</u> <u>Sh/al sh</u> x <u>al et/al et</u>	<u>al-m(pa-pu)</u> <u>Sh/al sh</u> x <u>al et/al et</u>
4	L I H Total	L I H Total
5	42 6 1 49 (86) (12) (2)	17 8 1 26 (65) (30) (4)
6	F ₂ progenies (unclassified genotypes)	
	 x <u>al et/al et</u>	
7	L I H Total	
8	140 6 12 158 (89) (4) (7)	

Figure 4A. Test of seed-set on the al-m(pa-pu) genotype having two different sources of cytoplasm (al-m(pa-pu) vs al sh); numbers in parentheses are percent of total

contamination since shrunken seeds (al sh/al sh) would not be expected from the cross al-m(pa-pu) Sh/al sh x al et/al et. From this same cytoplasmic source, an additional test was conducted in the following year to verify the earlier result. Unclassified genotypes of the F_2 seeds from 10 F_1 s having al sh cytoplasm (Figure 4A, column 2) were assayed for RSS effect by testcrossing with al et/al et males. Again, RSS effect was observed among the 140 ears (89%) (out of the total of 158 ears) which were of the class L type (Figure 4A, lines 7 and 8).

It can be concluded that the RSS effect is expressed by the al-m(pa-pu) genotype carrying either al-m(pa-pu) cytoplasm or al sh cytoplasm. The similarity of the RSS effect between the al-m(pa-pu) cytoplasm and al sh cytoplasm shows no difference in the two cytoplasms. If the control of RSS effect includes a cytoplasmic component, it is not specific to the original al-m(pa-pu) cytoplasmic source.

A contrary result was obtained when a cytoplasm from an unrelated W22 color converted line (line C) was examined in a similar type of cross. From the F_1 s of the cross Al Sh/Al Sh (line C) x al-m(pa-pu) Sh/al sh males, three genotypes, al-m(pa-pu) Sh/al-m(r) Sh, al-m(pa-pu) Sh/al-m-1 sh, and al-m(pa-pu) Sh/al sh, all with W22 (line C) cytoplasm, were developed using the following procedures (Figure 4B). The F_1 s, al-m(pa-pu) Sh/Al Sh (line C) were selfed (Figure 4B, column 1) or crossed by al sh/al sh males (Figure 4B, column

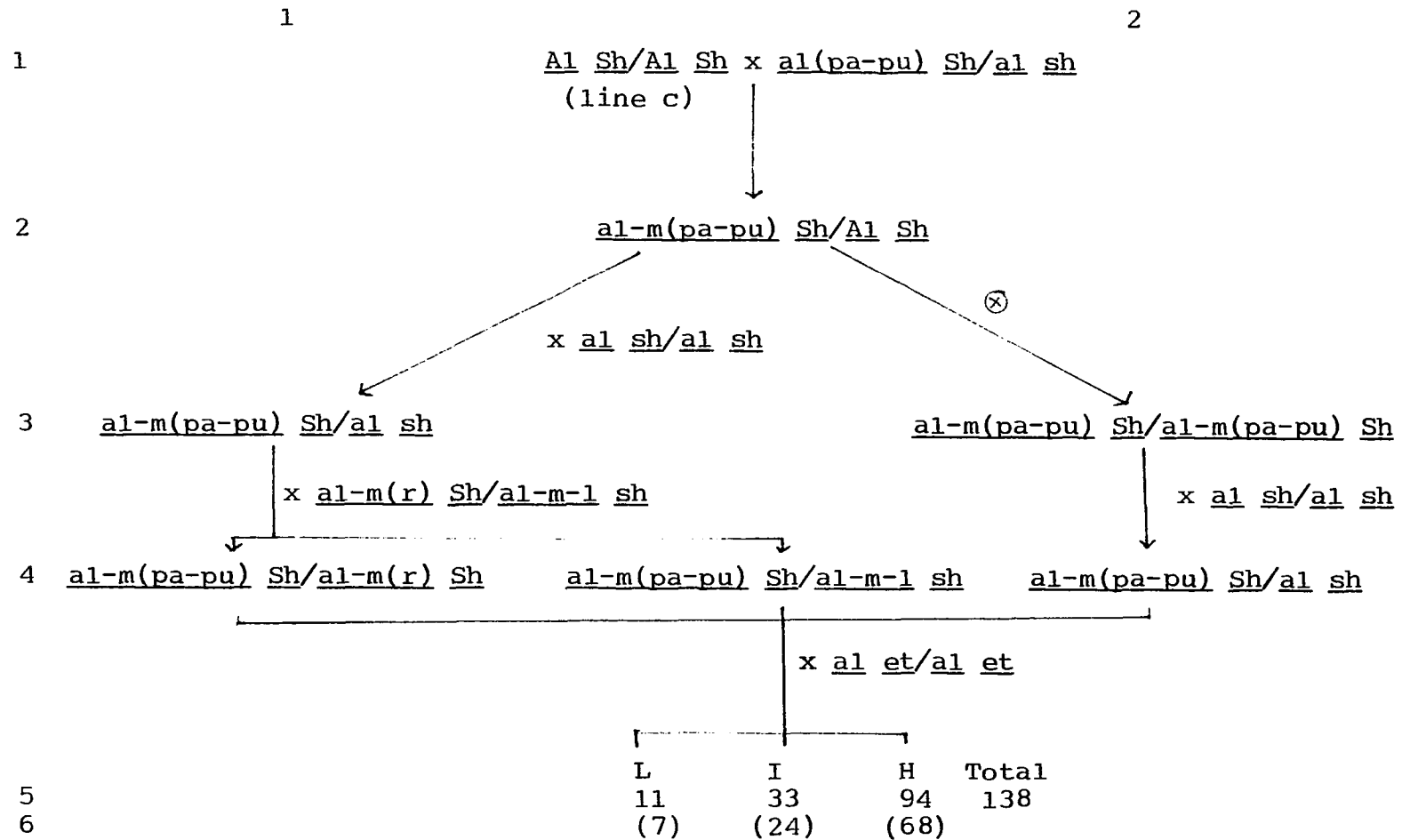


Figure 4B. Test of RSS effect on al-m(pa-pu) genotype having unrelated (line c) cytoplasm (numbers in parentheses are percent of total)

2) to obtain al-m(pa-pu) Sh/al-m(pa-pu) Sh al-m(pa-pu) Sh/al sh genotypes. The F_2 progenies (Figure 4B, column 2) were crossed by al sh/al sh and the resulting progenies derived from crosses with al sh/al sh subsequently were crossed by al-m(r) Sh/al-m-1 sh. It should be noted that this crossing procedure is the same as the procedure used in developing al-m(pa-pu) lines (Table 5A). Crosses with al-m(r) Sh/al-m-1 sh are those commonly used in the study of controlling elements in maize in order to test for the presence of En (Peterson, 1970).

The three genotypes illustrated in Figure 4B, line 4, were assayed for RSS effect by testcrossing with al et/al et males. It is evident that the al-m(pa-pu) derivatives carrying line C cytoplasm do not yield RSS effect (Figure 4B, line 5). These results indicate that with the same genotypes as in the crosses with al sh/al sh, the line C cytoplasm can be differentiated from the cytoplasm of the al-m(pa-pu) and the al sh lines in expressing the RSS effect.

These tests (Figures 4A and 4B) support the conclusion that the RSS effect results from an interaction of a genotype with a specific cytoplasm. Both the al-m(pa-pu) and al sh cytoplasm are similar in complementing the al-m(pa-pu) genotype in expressing the RSS effect. In contrast, the line C (W22 color converted line) cytoplasm does not similarly complement the al-m(pa-pu) genotype in the expression of the RSS effect.

Now that the cytoplasm is implicated by these tests to have a role in RSS control, the second and third possibilities of chromosomal factor(s) controlling the RSS effect will be examined as follows.

2. Test of the role of the al-m(pa-pu) allele in the control of the RSS

In maintaining the al-m(pa-pu) lines, the al-m(pa-pu) allele has been continually selected. The incompatibility crosses are al-m(pa-pu) females or derivative alleles x al et males and thus involve the al-m(pa-pu) allele. It is possible that the al-m(pa-pu) allele itself or a gene closely linked to it is responsible for the control of the RSS effect.

To test this possibility, pale and purple spotting aleurone kernels [al-m(pa-pu) Sh/al sh] were selected from the remnant kernels of 1970 1457-3 derivatives on which RSS was confirmed (Table 5B). These kernels were planted and 10 plants were selfed. The selfed progenies from these plants were separated into two groups according to aleurone color. Kernels with pale and purple spotting aleurone color possess the al-m(pa-pu) allele in either the homozygous or heterozygous condition [al-m(pa-pu) Sh/al-m(pa-pu) Sh or al-m(pa-pu) Sh/al sh]. The progeny kernels that were colorless and shrunken were homozygous al sh/al sh. (Since the a-sh distance is .25 linkage units apart, this kernel separation does test the al-m(pa-pu) segment.) These selected kernels were

tested for RSS effect by testcrossing with al et/al et males. The test included 126 plants of the genotypes that carried the al-m(pa-pu) allele and 29 plants of the genotype that did not carry the al-m(pa-pu) allele (al sh/al sh). The results (Figure 5, Part 1) show that RSS is expressed in both groups [with al-m(pa-pu) vs without al-m(pa-pu)]. In the first group [with al-m(pa-pu)], 114 ears (90%) fall into class L (<25 k/ear), and thus agrees with previous results where the RSS effect was observed (Table 5B). In the second group (al sh/al sh), 25 ears (86%) fall into class L and, again, the result is consistent with previous values showing the RSS effect.

A parallel test was conducted using, again, the remnant kernels of O 1457-3 derivatives (confirmed RSS; Table 5B) that were progeny of the cross al-m(pa-pu) Sh/al sh x al sh/al sh. In this test, both pale purple spotting-round [al-m(pa-pu) Sh/al sh] and colorless shrunken (al sh/al sh) kernels were planted and testcrossed by al et/al et males for the expression of the RSS effect (Figure 5, Part 2). The results (Figure 5, Part 2, lines 4 and 5) show that the RSS effect appears in both tested genotypes [with and without the al-m(pa-pu) allele]. Out of a total of 152 progeny ears from the al-m(pa-pu) Sh/al sh source, 128 ears (84%) fall into class L, while 63 out of the total of 69 ears (91%) of al sh/al sh source fall into the same class.

From these results (Figure 5, Parts 1 and 2), it can be

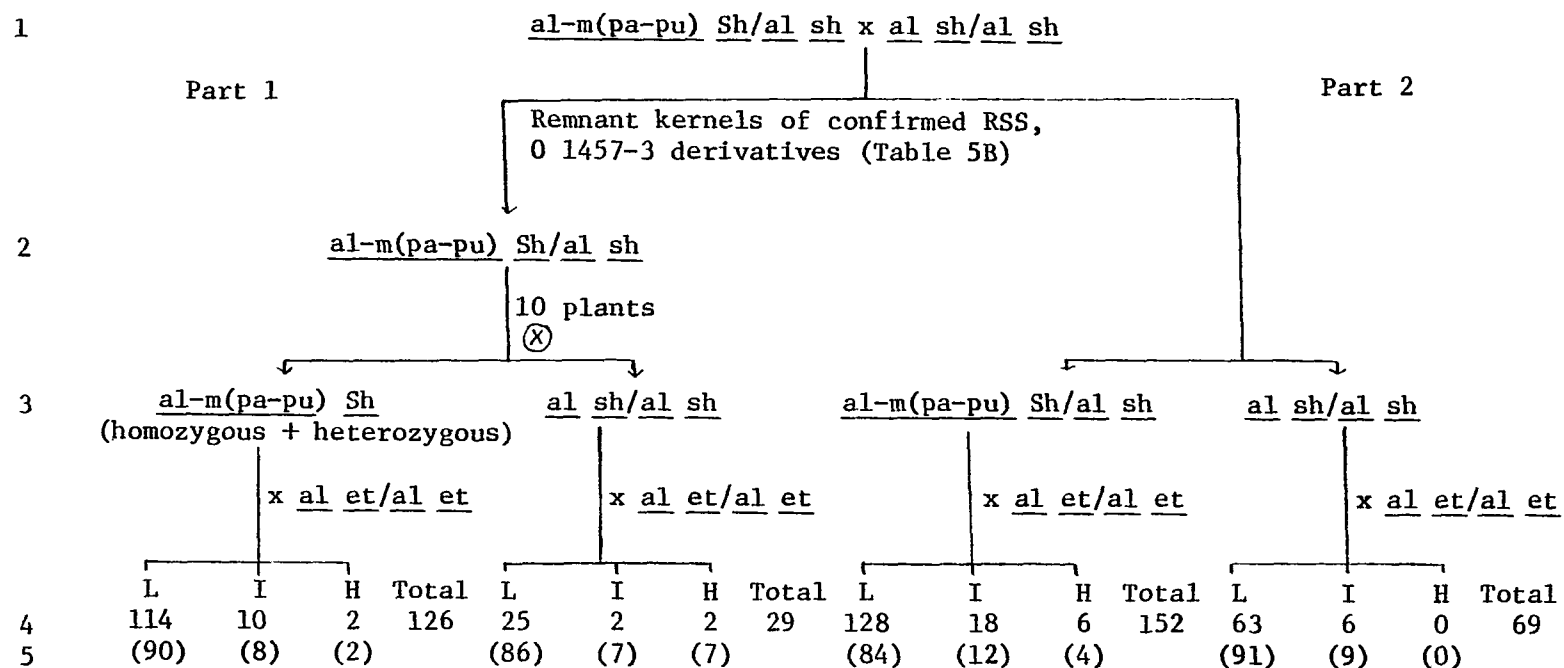


Figure 5. Test of RSS effect (x al et/al et males) of genotypes carrying al-m(pa-pu) and Sh alleles versus those that do not carry al-m(pa-pu) and Sh alleles (numbers in parentheses are percent of total)

concluded that the RSS effect is independent of the al-m(pa-pu)-Sh chromosome segment. This conclusion is based on the consistency of the appearance of the RSS effect in both genotypes, those with and without the al-m(pa-pu)-Sh chromosome segment. Further, since the RSS effect does not differ between the parental generation (Figure 5, Part 2) and the derivative F_2 generation at the same level (90%) (Figure 5, Part 1), it is implied that there is no segregation of the RSS controlling factor among the F_2 progenies. If segregation has occurred, it has not been detected.

3. Test of the role of Gal^S allele in the control of the RSS effect

Nonreciprocal cross-sterility in maize has been reported by Schwartz (1950) and Nelson (1952). As described in the literature review, the sterility, in this case, was shown to be associated with the Gal^S allele of the Gal locus of chromosome 5. In the present study of the RSS effect, the incompatibility resulting from the crosses of al-m(pa-pu) Sh/ al sh x al et/al et males yield ears with a reduced seed set which reflect a type of sterility. For this reason, there is a possibility that the RSS effect in the al-m(pa-pu) lines is also associated with the Gal^S allele.

According to the finding of Schwartz (1950) and Nelson (1952), Gal^S/Gal^S , Gal^S/Gal or Gal^S/gal silks reject gal pollen. With this finding as a model, it is assumed that the al-m(pa-pu) lines could contain either Gal^S/Gal^S ,

Gal^S/Gal, or Gal^S/gal genotypes and the al et/al et genotype is gal/gal.

The al sh/al sh must be similar in genotype with respect to RSS. This assumption is based on two observations. First, the al-m(pa-pu) lines and the al sh/al sh lines are compatible. Secondly, several generations of recurrent crossing al-m(pa-pu) Sh/al sh by al sh/al sh does not dilute the RSS effect among the progeny (Table 5A and Table 5B). Under these conditions, it would be expected that al sh/al sh tester must be carrying the same Gal^S allele as the original al-m(pa-pu) lines. With this deductive reasoning, it can be assumed that the RSS effect arises from the cross al-m(pa-pu) Sh/al sh, Gal^S/Gal^S x al et/al et, gal/gal male.

To test this model of the Gal^S involvement in RSS expression, crosses between the two principal parents of the RSS effect were made using, in this case, the al et/al et as the female parent and al-m(pa-pu) Sh/al sh as the male parent. Since crossing al et/al et x al-m(pa-pu) Sh/al sh males results in NSS (Table 4), it can be assumed that al et/al et is gal/gal and al-m(pa-pu) Sh/al sh is Gal^S/Gal^S. This follows from Nelson's (1952) findings that gal/gal silks do not reject Gal^S pollen. The resulting F₁s of the above cross [al et/al et x al-m(pa-pu) Sh/al sh] are expected to be Gal^S/gal which normally rejects gal pollen. By crossing these F₁s by al et/al et males, the resulting ears should then show the RSS effect. The results of these crosses,

however, do not agree with the expectation based on the projected model of anticipated Gal segregation (Figure 6). The progeny ears are clearly of the NSS type, not the RSS type, effect. From a total of 210 progeny ears in this test, only 8 ears (4%) are in class L. This is an expected frequency of ears with low seed set normally seen in compatible crosses, thus supporting the appearance of NSS type progeny from the F_1s x al et/al et crosses.

These results would support the rejection of the gal^S model as the basis for the RSS effect arising from the crosses of al-m(pa-pu) Sh/al sh x al et/al et males.

4. Test of the possibility of a chromosomal-cytoplasmic interaction as the basis for control of the RSS effect

From the presented evidence, it is concluded that the RSS effect is not under the control of either a chromosomal factor alone, or a cytoplasm alone. It is necessary, therefore, to examine the last possibility that the RSS effect results from a chromosomal-cytoplasmic interaction in the al-m(pa-pu) lines. Support for this assumption is derived from evidence illustrated in Figure 7. Here, the incorporation of the al et/al et genomes into the al-m(pa-pu) lines (retaining the original cytoplasm) that originally gave only RSS, now changes to NSS, when they are assayed with the al et/al et males.

The rationale of the test is as follows. If the cytoplasm alone from the al-m(pa-pu) Sh/al sh parent is

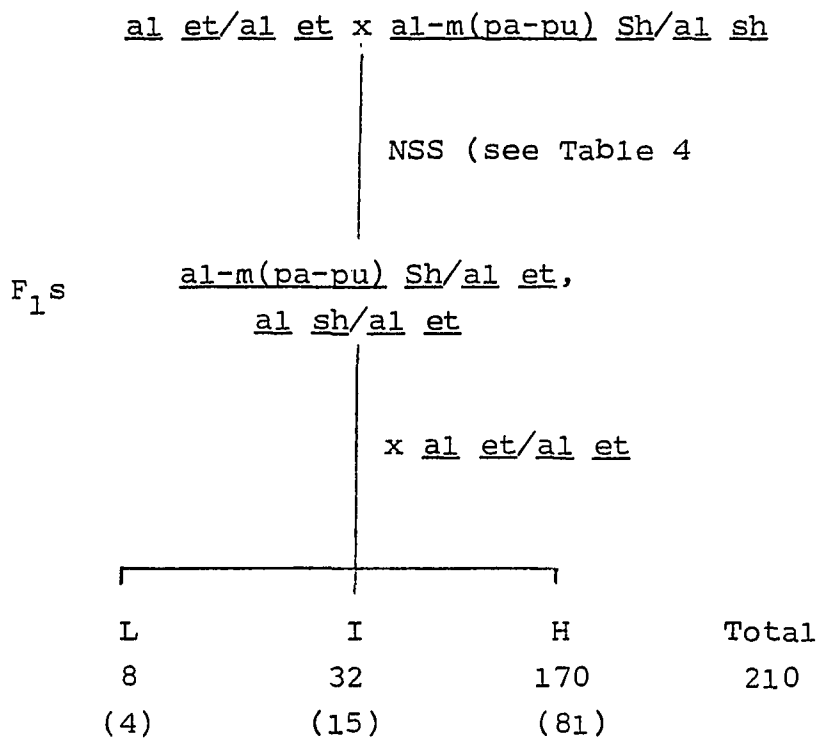


Figure 6. Test of seed-set on the F_1 s from the cross originating as al et/al et x al-m(pa-pu) Sh/al sh (numbers in parentheses are percent of total)

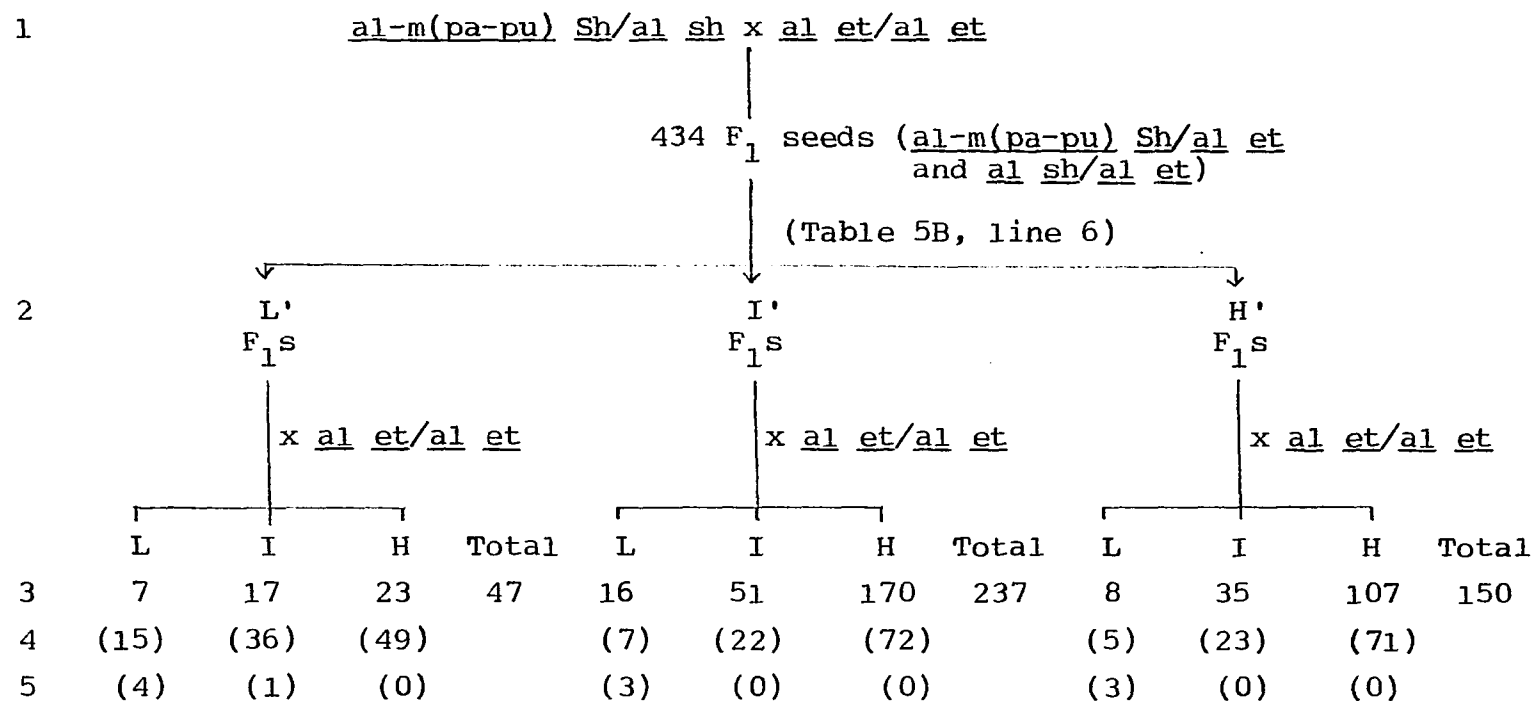


Figure 7. Test of RSS effect on 3 different classes (L'=<25 k/ear, I'=26-200 k/ear, H'=>200 k/ear) of F₁s of the cross al-m(pa-pu) Sh/al sh x al et/al et (the prime symbol refers to progeny types derived from crosses which gave rise to progenies that were further tested for RSS) (line 4 numbers in parentheses are percent of total, line 5 numbers in parentheses are number of ears without etched kernels)

responsible for the RSS effect, then all genotypes containing this cytoplasm should express the RSS effect. The genotypes used in this test were al-m(pa-pu) Sh/al et and al sh/al et. These genotypes were the F_1 progenies derived from the crosses al-m(pa-pu) Sh/al sh x al et/al et males that retained the original al-m(pa-pu) cytoplasm. These crosses expressed the RSS effect as described in Table 5B (line 6). A total of 434 F_1 seeds from the three classes of ears ($L' = L$, $I' = I$, and $H' = H$) was planted and these plants were testcrossed by al et/al et males to test for the expression of the RSS effect (Figure 7, line 2). The data (Figure 7, line 4) show that F_1 s from all three classes (L' , I' , and H') yielded NSS. When the distributions of the ears among class L , I , and H (Figure 7, line 3) are compared using a chi-square testing, it is evident that the ear-distribution in the F_1 s of class L' is statistically different from the ear-distributions in the F_1 s of the other two classes. This difference is due to the higher percentage of class L in the F_1 s of class L' than those in the F_1 s of class I' and H' (15% vs 7 and 5%, respectively) (Figure 7, line 4). Although 15% is not comparable to the 80-90% class L seen in the RSS effect, there is a basis for further consideration of this segregation. It is suspected that the source of some kernels among the F_1 s derived as class L' type progeny might have originated from contaminant pollen of the al-m(pa-pu) Sh/

al sh parent by selfing rather than al et pollen in the parental cross (Figure 7, line 1).

To confirm this presumed origin of these F_1 seeds, the progeny ears (Figure 7, line 3) were analyzed for parental authenticity. The presence of etched (et/et) kernels would confirm that those particular F_1 s were derived from the specified cross, al-m(pa-pu) Sh/al sh x al et/al et males (Figure 7, line 1). If etched kernels were absent among the progeny ears, those particular F_1 s were not the products of the specified cross but derived from a contaminated cross.

Out of 47 progeny ears of the F_1 s of class L', five were without the expected etched marker (Figure 7, line 5). Four of these (5) ears were from among the 7 of the class L group. Only 3 of the 237 ears of class I' and 3 of the 150 ears of class H' lacked etched marker (Figure 7, line 5).

It seems that most of the F_1 s (Figure 7, line 2) were derived from the specified cross [al-m(pa-pu) Sh/al sh x al et/al et male], although of the 11 ears (11/434) originating from contamination, five were from the L' class. Contamination is more likely to occur among class L type ears leading to RSS than in NSS type ears. This is supported by the higher percentage of class L in the F_1 s of class L' than those in the F_1 s of class I' and H'.

These results lead to the conclusion that the RSS effect that is evident among the progeny with the original cytoplasm of the al-m(pa-pu) parent universally disappears in the F_1

genotypes that are al-m(pa-pu) Sh/al et or al sh/al et, whether they are L', I', or H' types. The results of the crosses F_1 s x al et/al et males reflect a change to NSS.

It can be further concluded from the evidence that the RSS effect depends on the chromosomal constitution of the al-m(pa-pu) lines complementing the al-m(pa-pu) cytoplasm. The inclusion of the al et/al et genotype into the F_1 s of the cross al-m(pa-pu) Sh/al sh x al et/al et leads to the disappearance of the RSS effect.

5. Test of the change from RSS to NSS resulting from the incorporation of the genomes from al et/al et parent

Since the incorporation of the genomes from al et/al et parent into the F_1 s of the crosses al-m(pa-pu) Sh/al sh x al et/al et males changes the expected RSS expression of these F_1 s to NSS, a question arises whether the RSS to NSS change is permanently altered.

To answer this question, a series of crosses, illustrated in Figure 8, was examined. The BC_1 progenies from the cross F_1 s [from al-m(pa-pu) Sh/al sh x al et/al et] x al et/al et males showed NSS (Table 6, line 3) when they were crossed by al et/al et males. The subsequent BC_2 progenies also showed NSS in a similar assay with al et/al et males (Table 6, line 4). These observations indicate that once the al-m(pa-pu) lines are crossed by al et/al et, the change from RSS is altered to NSS. However, the suggested

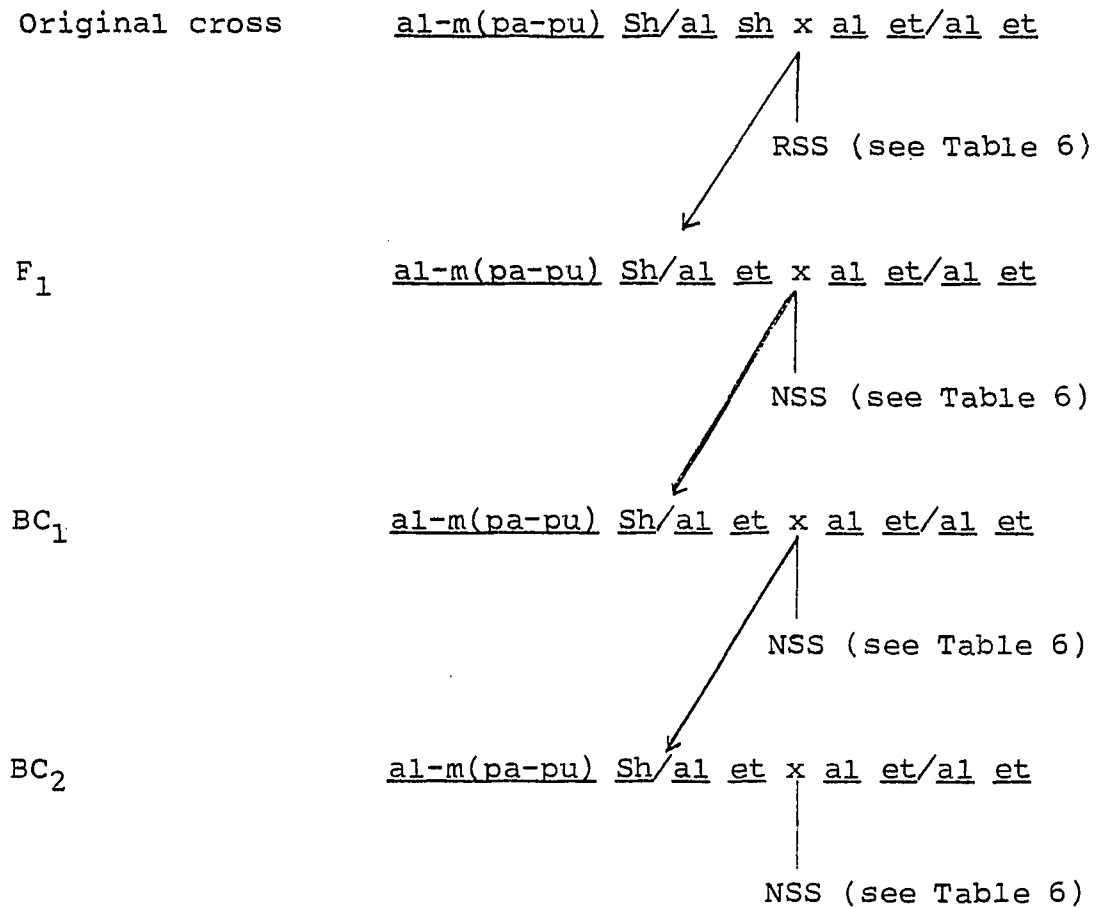


Figure 8. Observed NSS (when crossed by al et/al et males) in F₁s, BC₁s and BC₂s of the cross al-m(pa-pu) Sh/al sh x al et/al et when al et/al et was the recurrent parent

Table 6. Observed seed-set (arising from crosses with al et/al et males as the recurrent parent) on F₁s, BC₁s, and BC₂s of the cross al-m(pa-pu) Sh/al sh x al et/al et (Figure 8)

1		2	3	4	5	6
Generation of seed source		Genotype tested	Number of ears of each class (%)			Total
			L<25 k/ear	I=26-200 k/ear	H>200 k/ear	
1	0	<u>al-m(pa-pu)</u> <u>Sh/al</u> <u>sh</u>	98 (86)	15 (13)	1 (1)	114
2	F ₁ ^a ns ^b	<u>al-m(pa-pu)</u> <u>Sh/al</u> <u>et</u>	24 (6)	86 (22)	277 (72)	387
3	BC ₁ ns	<u>al-m(pa-pu)</u> <u>Sh/al</u> <u>et</u>	5 (3)	52 (28)	128 (69)	185
4	BC ₂ ns	<u>al-m(pa-pu)</u> <u>Sh/al</u> <u>et</u>	5 (7)	29 (41)	36 (51)	70

^aSeed from class I and H of previous crosses (Figure 7).

^bns = nonsignificant difference (among F₁, BC₁ and BC₂).

permanency of this change may be misleading because many of the genes of the al-m(pa-pu) lines are being replaced by the genetic material from al et/al et because the al et/al et line is used as the recurrent parent.

Can the interacting units contributing to the RSS effect be recovered? To answer this question, the lines that have been derived from crosses with al et/al et as the female parent, namely, the progeny from the cross al et/al et x al-m(pa-pu) Sh/al sh (Figure 6), were used. This progeny possesses al et/al et cytoplasm instead of the original al-m(pa-pu) cytoplasm and as such the cytoplasmic factor must be brought into consideration. In tests of these F_1 progenies for the RSS effect (Figure 6) by crossing with al et/al et males show NSS. If RSS can be recovered in the progenies of these F_1 s, it would indicate that al et/al et cytoplasm can complement with particular genotypes and express the RSS effect.

The test was conducted as illustrated in Figure 9. Starting with an al et/al et cytoplasmic source, selfing of the F_1 plants yielded F_2 seeds with an assortment of genotypes including al-m(pa-pu) Sh/al-m(pa-pu) Sh, al-m(pa-pu) Sh/al et. These genotypes were identified by the sectoring pale-purple aleurone color. Seventy plants were obtained from these F_2 seeds and tested for RSS effect by crossing with al et/al et males. The results are shown in Figure 9.

The RSS effect was recovered among the F_2 progenies from

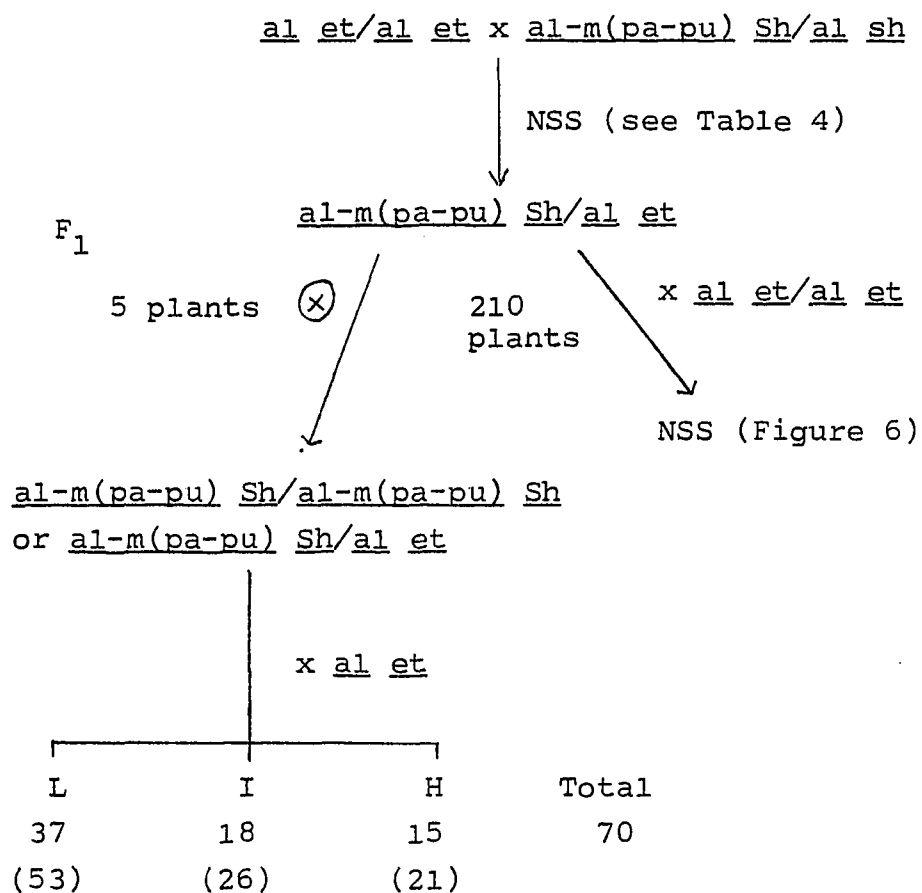


Figure 9. Test of RSS (when crossed by al et/al et males) on the F_2 progenies of the cross al et/al et x al-m(pa-pu) Sh/al sh (numbers in parentheses are percent of total)

the original cross of al et/al et x al-m(pa-pu) Sh/al sh. It is noted that out of a total of 70 ears, only 37 ears (53%) fall into class L--a value considerably lower than that expected (80-90%) in the expression of the RSS effect. It is postulated that this lowered value of class L to be influenced by the assortment of the genetic materials that originated from al et/al et parent that interfere with the RSS expression in the source F_1 progenies of the original cross. This finding then supports the contention that the al et/al et cytoplasm can complement with a particular genotype leading to the RSS expression.

D. Genetic Component of the Pollen Parent Contributing to the Incompatibility Reaction

Two types of pollen parents have been identified with respect to incompatibility that results in the RSS effect among al-m(pa-pu) lines. The compatible type that includes al sh/al sh, al-m(r)/al-m-l, and Al et/al sh testers yields NSS-type progenies when it is used as pollen parent in a cross with al-m(pa-pu) female parent (Table 7, lines 1 and 2). The incompatible type includes al et/al et, Al et/Al et, and c sh1 wx/c sh1 wx testers. These testers when used as pollen parents in crosses with al-m(pa-pu) female parents show incompatibility reaction which results in RSS expression (Table 7, lines 5 and 6).

The first set of data (compatible group; Table 7,

Table 7. Test of two groups (compatible vs incompatible) of pollen parents affecting seed-set on al-m(pa-pu) lines [al-m(pa-pu) Sh/al sh x pollen parent]

Pollen parent		Number of ears of each class (%)			Total
		L=<25 k/ear	I=26-200 k/ear	H=>200 k/ear	
<u>Compatible (ns1)^a</u>					
1	<u>al sh/al sh</u>	6 (6)	35 (32)	67 (62)	108
2	<u>al-m(r)/al-m-1</u>	4 (4)	17 (17)	77 (78)	98
3	<u>Al et/al sh</u>	3 (3)	7 (8)	77 (89)	87
<u>Incompatible (ns2)^b</u>					
4	<u>al et/al et</u>	176 (89)	16 ((8)	6 (3)	198
5	<u>Al et/Al et</u>	70 (91)	4 (5)	3 (4)	77
6	<u>c sh1 wx/c sh1 wx</u>	118 (91)	9 (7)	3 (2)	130

^ans1 = nonsignificant difference within compatible group.

^bns2 = nonsignificant difference within incompatible group.

lines 1, 2, and 3) was obtained in 1979. In these crosses, the remnant seeds of al-m(pa-pu) lines with confirmed RSS effect in crosses with al et/al et in the previous year (1978) were used. They were planted in 30 rows, 12 seeds per row with 2 replications and pollinated by al sh/al sh, al-m(r)/al-m-1, and Al et/al sh males. Each of the pollen parents were used in the pollination of two plants per row. The residual plants in each row were crossed by al et/al et pollen as a control. In some rows with less than 6 plants, only 1 plant was used for each type of pollen, except for the al et/al et control. The resulting ears were classified according to the number of seed-set per ear as described in the Materials and Methods. The results from the 2 replications were combined since they appeared to be similar. All these types of pollen parents yielded NSS when they were crossed on to the al-m(pa-pu) females. At the same time, the control crosses [al-m(pa-pu) females x al et/al et males) showed RSS effect (Table 3A, line 5). In this first set of data (Table 7, lines 1, 2, and 3), the compatibility between the al-m(pa-pu) females and al sh/al sh or al-m(r)/al-m-1 or Al et/al sh males is confirmed.

The second set of data (Table 7, lines 4, 5, and 6) was obtained from the crosses in 1980. This test again confirmed al-m(pa-pu)-RSS-yielding lines were used as the female parent. The rationale for using the Al et/Al et genotype as a pollen parent was to test the possibility that the al allele itself

or a factor located between the al and et alleles in the al et/al et tester was responsible for the incompatibility reaction. Thus, this test with A1 et pollen would yield different results from the al et pollen, that is, it would yield NSS. Since A1 et/A1 et and al et/al et were derived from the same source (1972 792-25 A1 et/A1 et; Table 2A), they were expected to have a similar background. If it was not the al allele itself or a factor in the al-et region that caused incompatibility reaction, the A1 et/A1 et pollen parent would be expected to yield RSS when it was crossed on to the al-m(pa-pu) females.

Another genotype used in this test of pollen parent was the c sh1 wx/c sh1 wx tester. The rationale of using this tester was that if the incompatibility reaction in the pollen was specific to only the lines that were derived from A1 et/A1 et stock, the c sh1 wx/c sh1 wx line would give NSS since it would not be incompatible with the al-m(pa-pu) females.

As in the compatible crosses, the al-m(pa-pu) seeds were also planted in 30 rows with 12 plants per row, in 2 replications. The pollen from al et/al et, A1 et/A1 et and c sh1 wx/c sh1 wx plants was each crossed on to 3 plants of each row. The rest of the plants in these rows were crossed by the al sh/al sh pollen parent as the control crosses.

It was observed that the crosses between the al-m(pa-pu) females and al et/al et or A1 et/A1 et, or c sh1 wx/c sh1 wx

males consistently yielded RSS (Table 7, lines 4, 5, and 6). The results from the 2 replications were combined because they appeared to be the same.

It can be concluded that it is not the a1 allele itself or even the et allele or any factor located in the a1-et region that is responsible for the incompatibility reaction in the pollen parent. Both a1 et/a1 et and A1 et/A1 et yielded RSS when they were crossed with the a1-m(pa-pu) females.

The fact that c sh1 wx/c sh1 wx line, when used as pollen parent, also showed an incompatibility effect could be explained by the presumption that this tester shared a common factor with the a1 et/a1 et and A1 et/A1 et parents. This presumption is reasonable because many of these tester lines originated as a common series in the original marker line development at Cornell University (Dr. P. A. Peterson, Dept. of Agronomy, Iowa State University, personal communication).

In the maintenance of the a1 et/a1 et, A1 et/A1 et, and c sh1 wx/c sh1 wx tester lines, the plants were selfed and sibbed for many generations. This operation is expected to bring most genes including the one(s) that is (are) responsible for the incompatibility reaction in the pollen into homozygous condition. The question whether the allele which is responsible for the incompatibility reaction is dominant or recessive is yet to be answered.

The compatible crosses must include parents that lack the incompatibility factor since the a1 sh/a1 sh and

al-m(r)/al-m-1 testers yield NSS when they are crossed with al-m(pa-pu) females. If the incompatible testers (al et/
al et, Al et/Al et, and c sh1 wx/c sh1 wx) possess homozygous recessive alleles for incompatibility the compatible testers (al sh/al sh and al-m(r)/al-m-1) must possess homozygous dominant alleles for compatibility. Following the same reasoning, it could be expected that, if the incompatibility factor is dominant, the incompatible testers carry homozygous dominant alleles, and the compatible testers must carry homozygous recessive alleles.

Crossing a compatible tester with an incompatible tester should give a heterozygous genotype (F_1) for the incompatibility factor. Such F_1 genotype was developed from the cross Al et/Al et (incompatible) x al sh/al sh (compatible), and it was represented by the Al et/al sh tester. When the pollen from these F_1 s were crossed on to the al-m(pa-pu) females, the resulting ears showed NSS (Table 7, line 3).

This result indicates that the compatible condition is dominant to the incompatible condition (compare line 3 with line 5, Table 7).

There is yet a question of the control of the incompatibility reaction expressed by the al et/al et parent, that is, whether it is sporophytic or gametophytic. From the heterozygote, Al et/al sh, two types of pollen grains, incompatible and compatible, would be produced. If the control is gametophytic, it could be expected that only half of

the pollen grains produced by A1 et/a1 sh would affect fertilization of the a1-m(pa-pu) female parent. On the other hand, if the control of incompatibility condition in the pollen parent is sporophytic, each type of pollen produced by A1 et/a1 sh parent will be functional on the a1-m(pa-pu) female parent.

In an attempt to identify the level of control of the incompatibility in the pollen parent, the available data from the cross using A1 et/a1 sh pollen were reexamined. The segregation ratios of the phenotypic markers were recorded among the progeny of the cross a1-m(pa-pu) derivatives x A1 et/a1 sh. If incompatibility is under gametophytic control, and the incompatibility factor is linked to the A1-Sh-et region in the heterozygote, A1 Sh et/a1 sh Et, a reduction or disappearance of colored (C1), round (Sh) and etched (et) kernels would be expected. (Due to the ambiguity in identifying the etched phenotype, only colored (C1):colorless (c1) and round (Sh):shrunken (sh) ratios were recorded.) Data in Table 8 represent a sample of the phenotypic ratios observed on the ears of the cross a1-m(pa-pu) derivatives by the A1 Sh et/a1 sh Et pollen parent.

The evidence (Table 8) indicates that the transmission of the marker alleles follows a normal Mendelian segregation of C1:c1 and Sh:sh as expected. The lack of discrimination against the colored or round kernels leads to the conclusion that the incompatibility factor is not linked to the A1-Sh-et

Table 8. Observed values and expected ratios of C1:cl, Sh:sh of ears from the cross al-m(pa-pu) derivatives (females) (Table 3) x Al et/al sh (males)

			Ratios								Chi-square values	
Genotype of female parent	Genotype of male parent	Individual ear	Observed				Expected					
			<u>C1</u>	<u>cl</u>	<u>Sh</u>	<u>sh</u>	<u>C1</u>	<u>cl</u>	<u>Sh</u>	<u>sh</u>		
1	<u>al-m(pa-pu) Sh/al Sh</u>	<u>Al et/al sh</u>	9 1411-1	183	58	-	-	3	1	-	-	ns ^a
2			9 1412-6	235	81	-	-	3	1	-	-	ns
3			9 2845-10	205	75	-	-	3	1	-	-	ns
4			9 2906-5	198	77	-	-	3	1	-	-	ns
5	<u>al-m(nr) Sh/al sh</u>		9 1450-4	-	-	410	102	-	-	3	1	ns
6			9 2840-10	-	-	96	33	-	-	3	1	ns
7			9 2902-3	-	-	363	94	-	-	3	1	ns
8			9 2908-2	-	-	140	48	-	-	3	1	ns
9			9 2910-6	-	-	90	38	-	-	3	1	ns
10			9 2925-3	-	-	148	63	-	-	3	1	ns
11			9 2525-4	-	-	252	123	-	-	3	1	ns

^ans = nonsignificant difference.

region in Al Sh et/al sh Et pollen parent. This conclusion is based only on gametophytic control of incompatibility.

Alternatively, if the incompatibility reaction is sporophytically controlled (by the dominance of al sh/al sh parent over Al et/Al et parent), then every pollen grain produced by Al et/al sh parent would be functional. The ratios of Cl:c1 or Sh:sh would always be as expected in the Mendelian segregation irrespective of the linkages to the A-Sh-et region. The available data (Tables 7 and 8) do not provide conclusive evidence to establish the level of the control of the incompatibility reaction expressed in al et/al et as to whether it is sporophytic or gametophytic. A further experimentation has been planned in investigating this particular question. The diagram in Figure 10 is used as a guideline for this plan.

Since the compatible condition is dominant to the incompatible condition (the Al et/al sh pollinating results from Table 7), it can be postulated that the compatible tester, al sh/al sh, has a homozygous dominant compatibility factor designated \pm/\pm , the incompatible tester, Al et/Al et, has m/m and their F_1 , Al et/al sh, has m/\pm . If the control of incompatibility reaction is gametophytic, then only \pm pollen effects fertilization. And, if the control of incompatibility reaction is sporophytic, both m and \pm pollen would effect fertilization.

Considering first gametophytic control (Figure 9), the crosses al-m(pa-pu) Sh/al sh, \pm/\pm x Al et/al sh, m/\pm would

yield only \pm/\pm type of progenies since only \pm pollen effects fertilization. But, if the sporophytic control is operating, the above crosses would yield both \pm/\pm and \pm/\underline{m} progenies, since both \pm and \underline{m} pollen effect fertilization.

To identify whether the progenies of al-m(pa-pu) females x Al et/al sh males are of only \pm/\pm or \pm/\pm and \pm/\underline{m} types, they must be selfed or crossed by the $\underline{m}/\underline{m}$ genotype (Al et/Al et). As was related in the gametophytic control system, all the progenies of the cross \pm/\pm x \underline{m}/\pm (male) are \pm/\pm . Selfing \pm/\pm or crossing \pm/\pm by $\underline{m}/\underline{m}$ yields \pm/\pm and \pm/\underline{m} , respectively. Both \pm/\pm and \pm/\underline{m} will be compatible with al-m(pa-pu) females as compatibility is dominant to incompatibility.

On the other hand, in the sporophytic control system, the progenies of the cross \pm/\pm x \underline{m}/\pm (male) are \pm/\pm and \pm/\underline{m} . Selfing and crossing these progenies by $\underline{m}/\underline{m}$ (Al et/Al et) would yield \pm/\pm , \pm/\underline{m} , and $\underline{m}/\underline{m}$ or only \pm/\underline{m} and $\underline{m}/\underline{m}$, respectively. Either way, the $\underline{m}/\underline{m}$ genotype would be generated. Using these progenies (Figure 9, line 3) in the cross with al-m(pa-pu) females would be expected to show the incompatibility reaction in the ratios described in Figure 9, line 4.

Therefore, if the incompatibility reaction on RSS effect is recovered in the last step (cross with al-m(pa-pu) females), it could be concluded that the control of the incompatible condition in the pollen parent is sporophytic. If, on the other hand, the RSS effect is not recovered, the control of

incompatibility is gametophytic.

It should be noted, however, that the experimental scheme shown in Figure 9 is based on only a single incompatibility factor. If there is more than one factor affecting the incompatibility reaction, a similar scheme can be used, but a more complicated segregating ratio must be considered.

E. Preliminary Studies on Pollen Germination and Pollen-Tube Growth

As previously reported, the incompatibility reaction which leads to RSS expression occurs only when al-m(pa-pu) derivatives are used as female parents in the crosses with al et/al et or other pollen parents in the same (incompatible) group (Table 7). In the reciprocal crosses in which the al-m(pa-pu) derivatives are used as male parents, the RSS expression is not observed. These findings lead to the speculations that the incompatibility reaction leading to RSS expression in al-m(pa-pu) derivatives might be due to a number of different causes, such as inability of pollen from al et/al et (and the like) to germinate on the silks of al-m(pa-pu) lines, or inability of the pollen-tubes to grow in the silks, or inability of these tubes to reach the ovules, or inability of the pollen tube to penetrate the embryo sac, or inability of the fertilization to take place, or inability of the particular zygote development to occur inside the ovary

of the maternal parent.

At present, these speculations have not been thoroughly investigated. However, a number of preliminary studies have been made in the area of pollen germination and pollen-tube growth.

1. In vitro pollen germination

With respect to the compatibility with the al-m(pa-pu)-derived female parent, there are two types of pollen parents: compatible and incompatible (Table 7). The question is whether there is an abnormality in pollen germination or pollen-tube growth associated with the pollen from the incompatible source. To answer this question, an in vitro pollen germination procedure was conducted as described previously in the Materials and Methods.

The observations were made in in vitro germination of the pollen grains from al et/al et plants (incompatible type) and from al-m(pa-pu) plants (self-compatible type). These observations were made under a light microscope immediately after the preparation of slides. Pollen grains started to absorb liquid from the medium and pollen-tube growth was initiated in approximately 3 to 5 minutes and continuing on in the medium for 30 to 40 minutes at which time the pollen tubes started bursting (Figure 11).

The pollen from both sources (incompatible and compatible) germinated in the liquid medium. The pollen-tube growth was

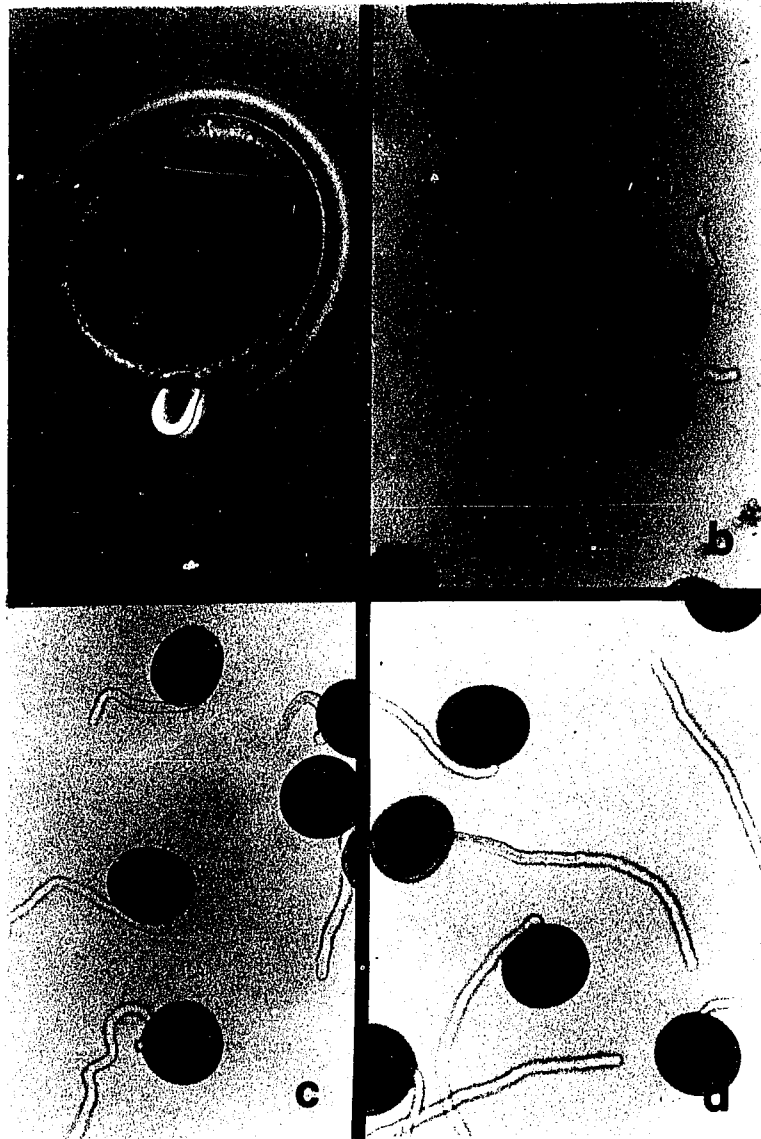


Figure 11. In vitro pollen germination and pollen-tube growth; pollen source: al et/al et, incompatible type when crossed with al-m(pa-pu) females (a = 2 min, b = 5 min, c = 10 min, d = 30 min)

normal for both sources. The rate of pollen-tube growth was not measured since the study was aimed only to determine if there is abnormality associated with pollen from the incompatible source (a1 et/a1 et).

It is not surprising, however, that the pollen from incompatible source (a1 et/a1 et) germinates and grows normally in vitro, since the pollen affects fertilization of female parents other than the a1-m(pa-pu) females (Table 3C). The in vitro study does confirm that the incompatibility reaction leading to the RSS effect in the a1-m(pa-pu) females results from the interaction between the a1-m(pa-pu) females and the pollen from a1 et/a1 et males.

2. In vivo pollen germination

Now that it is clear that the incompatibility leading to the RSS effect results from the interaction between the a1-m(pa-pu) females and the pollen from a1 et/a1 et parent, the questions of where and when the incompatibility reaction occurs follow. Since the interaction effect was absent in the in vitro pollen germination study, it is necessary to conduct an in vivo study.

In this study, samples of 24-hour pollinated silks were collected from a total of 9 plants of a1-m(pa-pu)-derived genotypes (6 plants have been pollinated by pollen from a1 et/a1 et male parent and 3 plants have been selfed). Germinated pollen grains on the silk surface were observed

under a light microscope. The pollen tubes could be seen coming out of the pores and growing for a distance on the silk surface until they enter the silk body, at which point the tubes could no longer be seen (Figure 12).

Squashing the silk tissues was not successful and, as a consequence, the part of the pollen tubes inside the silks could not yet be observed. With a more appropriate staining technique, the pollen-tube growth inside the silks can be observed.

The use of radioactive ^{32}P tracer technique for the study of pollen-tube growth in live silks has been suggested by House and Nelson (1958). However, the necessary equipment required for this technique is not available at this time.

Nevertheless, it can be concluded from this study that the incompatible-type pollen from al et/al et plants can germinate on the al-m(pa-pu) silks. This finding invalidates the possibility that incompatibility reaction occurs at the silk surface, and that the silk exudate is the inhibitor of pollen germination.

The question whether the incompatible-type pollen tubes can grow inside the al-m(pa-pu) silk tissues is still an unanswered question. If it could be determined that the incompatible-type pollen tubes do not grow inside the silks, whereas the compatible-type pollen tubes are able to grow, this would indicate that the site of the incompatibility reaction is in the silk tissues themselves. If it could be

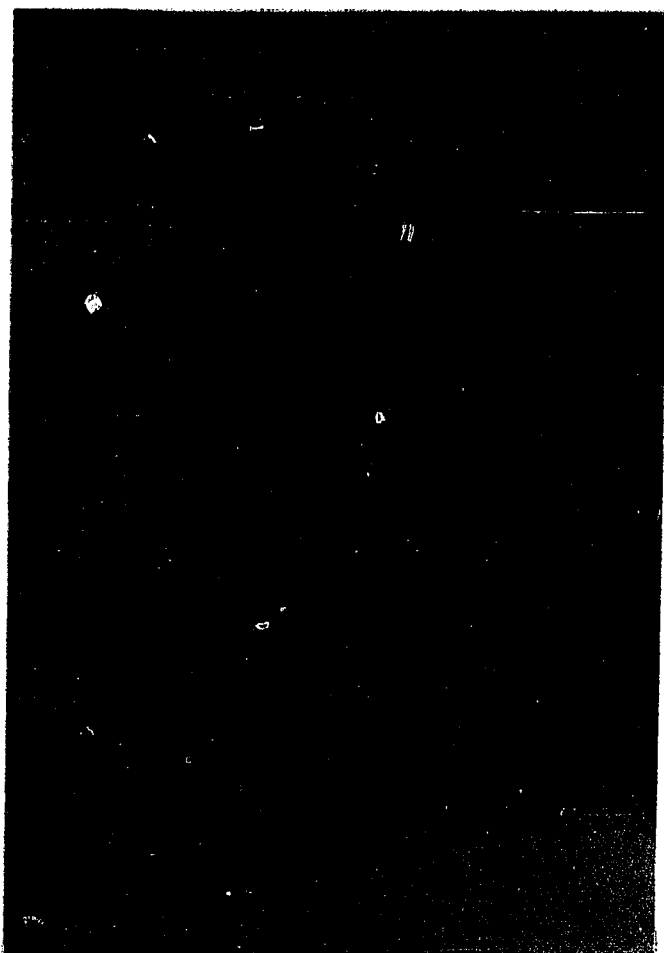


Figure 12. In vivo pollen germination on al-m(pa-pu) silks; pollen source: al et/al et, incompatible type when crossed with al-m(pa-pu) females

further determined that the incompatible-type pollen tubes grow normally inside the silk tissue until they reach the ovules, this would indicate that the incompatibility reaction may occur inside the ovules and may involve the fertilization process.

V. DISCUSSION

Incompatibility is a common phenomenon occurring in plant species including maize. Lewis and Crowe (1958) assumed that all self-compatible species originated from incompatible ancestors. These scientists argue that outbreeding which leads to new genetic combinations is important at a critical evolutionary stage when the environments are changing drastically. Probably after this crucial stage, when the environment is stabilized, outcrossing becomes non-essential. Only then did the inbreeding species emerge.

Cross-incompatibility in maize occurs between popcorns and dent corns. After an extensive investigation, Nelson (1952) found that in popcorns, cross-incompatibility (sterility) is a rule rather than an exception. According to Nelson, the genetic constitution of cross-sterile popcorns is $\underline{Ga}^S/\underline{Ga}^S$, while the genetic constitution of most dent corns is $\underline{ga}/\underline{ga}$. Nelson believes that the difference in the genetic constitutions of these two types of population might have a major significance in the evolution of the modern varieties of maize. He suggests that cross-sterile and normal popcorns may have had separate origin or diverged early in the course of their evolution. His further suggestion is that, if popcorns are progenitors of the modern maize type, these ancestral popcorn types could not have been among the cross-sterile varieties.

Cross-incompatibility condition in al-m(pa-pu) lines must have arisen from a mutational event which occurred in the course of the studies of controlling elements (Peterson, 1960, 1961). This event leads to a chromosomal change which interacts with the original al-m(pa-pu) cytoplasm leading to an incompatible female condition. This condition serves in screening against a specific type of pollen. In this case, the cross-incompatibility reaction is, therefore, specific for only a certain source of pollen.

The genetic control of incompatible condition in al-m(pa-pu) lines is rather complex when compared to that in popcorn (Nelson, 1952). Similar to the genetic control of hybrid dysgenic traits in D. melanogaster, the genetic control of cross-incompatibility in al-m(pa-pu) lines involves cytoplasmic-chromosomal interaction.

Like the hybrid dysgenic traits in D. melanogaster and self-incompatibility in other plant species, it will not be surprising if the incompatibility reaction in al-m(pa-pu) lines is found to be under the influence of other factors including polygenic modifiers and temperature. Further detailed investigations are necessary to answer all the questions that have arisen and will arise in the future.

VI. SUMMARY AND CONCLUSIONS

This is a study of a case of nonreciprocal cross-incompatibility between certain genetic lines of maize. Crosses between al-m(pa-pu) lines by al et/al et male testers yield ears with reduced seed-set (RSS). The reciprocal crosses, in which al-m(pa-pu) lines are used as male parents and the al et/al et testers as female parents, yield ears with normal seed-set (NSS). This incompatibility between al-m(pa-pu) females and al et/al et males is "incomplete" since the result is RSS instead of sterility.

The studies of the genetic control of the incompatible condition in the al-m(pa-pu) females show that the control involves cytoplasmic-chromosomal interaction. The cytoplasm of the al-m(pa-pu) lines is similar to the cytoplasm of the al sh/al sh testers in complementing specific genotypes leading to incompatibility reaction. The cytoplasm of line C (W22 colored converted line) does not, however, influence incompatibility.

The chromosomal factor involved in the interaction leading to incompatibility is not the al-m(pa-pu) allele itself nor does it involve the Gal^S allele which usually is associated with cross-sterility in maize (Nelson, 1952). This chromosomal factor is located in a position independent of the al locus.

The incorporation of the genomes from al et/al et parent

in the F_1 progenies of the cross al-m(pa-pu) females x al et/
al et males causes the change from RSS to NSS when the F_1 s
 are testcrossed by al et/al et males. The RSS effect can be
 recovered in the F_2 progenies. This result indicates that a
 factor in al et/al et genomes effects the change from RSS to
 NSS and this factor is segregating in the F_2 generation.

There are two types of pollen parents with respect to
 incompatibility with the al-m(pa-pu) females: compatible
 type and incompatible type. The F_1 progenies from the cross,
 incompatible type x compatible type, show compatibility ef-
 fect when they are crossed with al-m(pa-pu) females. This
 finding leads to the conclusion that the compatibility effect
 is dominant to the incompatibility effect.

The question whether the incompatibility effect involves
 sporophytic or gametophytic system is still unanswered. To
 derive some answers to this question, an experimentation
 scheme has been suggested.

The preliminary studies of pollen germination and
 pollen-tube growth show that pollen from the incompatible
 source (al et/al et) germinates and pollen tubes grow normal-
 ly in vitro. Since in the in vitro study, the interaction
 between the female tissue and the pollen is missing, the
in vivo pollen germination was examined. The results show
 that pollen from the incompatible source (al et/al et) also
 germinates and the pollen tubes grow normally on the silk

surface. However, once the tubes enter the silk body, they are not observable using the present staining technique. Unless a proper technique is used, these questions remain unanswered, whether the pollen tubes grow normally inside the silks, and whether the incompatibility occurs in the silks or in the ovules.

VII. BIBLIOGRAPHY

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